DEVELOPMENT OF A BROMOPEROXIDASE-BASED LEARNING CYCLE TO ENHANCE CONCEPTUAL UNDERSTANDING OF SECOND YEAR UNIVERSITY STUDENTS CONCERNING ENZYME KINETICS

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DEVELOPMENT OF A BROMOPEROXIDASE-BASED LEARNING CYCLE TO ENHANCE CONCEPTUAL UNDERSTANDING OF SECOND YEAR UNIVERSITY STUDENTS CORNCERNING ENZYME KINETICS

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ABSTRACT

This study employed the concepts of the learning cycle – a three-phase inquiry approach consisting of exploration, concept introduction, and concept application – to design learning activities for undergraduate science laboratory work in order to enhance Thai second year university students' understanding of enzyme kinetics. The laboratory activities designed for students were based on our own scientific work on one type of enzyme (bromoperoxidases) from the red seaweed *G. tangi* that has great promise for biotechnological applications.

In doing so, a simple detection for the enzyme activity in seaweed developed in the above mentioned research was used to engage students' interest in enzyme kinetics. Then the students were left to themselves to plan their own experiments concerning factors influence the rate of the enzyme's catalytic reaction. The results obtained were then compared and contrasted by the students themselves. In this learning cycle class, the teachers acted as facilitators to help students inquire effectively.

The effects of the designed activity on the students' understanding of enzyme kinetics and on their perceptions and preferences in the learning environment were examined. Five diverse data collecting methods were used for "triangulation". These were – achievement tests, questionnaires, interviews, classroom observation, and analysis of documents.

The analysis of the data from questionnaires, interviews, and classroom observation showed that the students were enthusiastic, happy and competitive. The students asked the teachers for help at the beginning but only briefly. They were more eager to collaborate and consult within the group to see whether their results were similar or different from others. Naturally the teachers had to conduct a wrap-up consensus session to clarify main points. The results also showed that, after performing these laboratory activities, the students seemed to follow the lecture better because they already had prior knowledge from their experiences in the laboratory part of the class. The results from the achievement tests and the analysis of students' documents such as concept maps, laboratory reports, posters as well as their submitted assignments showed that students had a better conceptual understanding of enzyme kinetics. Their post-test scores (36.96 ± 6.71) were significantly higher than their pre-test scores (25.25 ± 6.46) as was expected; however, students' knowledge retention was much better than expected.

KEYWORDS: ACTIVE LEARNING/ BROMOPEROXIDASE/ ENZYME /
LEARNING CYCLE / UNDERGRADUATE LABORATORY

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การพัฒนารูปแบบการเรียนรู้ปฏิบัติการเรื่องการทำงานของเอนไซม์โบรโมเปอร์ออกซิเดสเพื่อส่งเสริมให้นักศึกษาระดับปริญญาตรีชั้นปีที่ 2 มีความเข้าใจการทำงานของเอนไซม์

(DEVELOPMENT OF A BROMOPEROXIDASE-BASED LEARNING CYCLE TO ENHANCE CONCEPTUAL UNDERSTANDING ON ENZYME KINETICS OF SECOND YEAR UNIVERSITY STUDENTS)

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บทคัดย่อ

การศึกษาในครั้งนี้ได้นำเอาแนวคิดการจัดการเรียนรู้แบบ learning cycle มาจัดการเรียนรู้ในห้องเรียนปฏิบัติการวิทยาศาสตร์ในมหาวิทยาลัย เพื่อส่งเสริมให้นักศึกษาปริญญาตรีชั้นปีที่ 2 มีความเข้าใจการทำงานของเอนไซม์ โดยเน้นให้นักศึกษาสร้างความรู้ขึ้นเองผ่านกิจกรรม 3 เฟส คือ การสืบเสาะหาความรู้จากการทำปฏิบัติการ, การนำผลที่ได้จากการทำปฏิบัติการมาสร้างองค์ความรู้, และการนำความรู้ไปใช้ เนื้อหาในปฏิบัติการนี้เป็นการศึกษาเกี่ยวกับการทำงานของเอนไซม์โบรโมเปอร์ออกซิเดสที่สกัดได้จากสาหร่ายทะเลสีแดง *G.tangi* ซึ่งถูกพัฒนาขึ้นมาจากงานวิจัยทางวิทยาศาสตร์อันเป็นส่วนหนึ่งในการศึกษาครั้งนี้

ในห้องเรียนปฏิบัติการ ได้ใช้กิจกรรมการทดสอบเอนไซม์ในสาหร่ายทะเลอย่างง่ายๆ ในการกระตุ้นให้นักศึกษาเกิดความสนใจใคร่รู้ จากนั้นนักศึกษาจะได้ออกแบบการทดลองภายในกลุ่มของตนเองเพื่อศึกษาปัจจัยที่มีผลต่อการทำงานของเอนไซม์ นักศึกษาแต่ละกลุ่มเป็นผู้กำหนดปริมาณและค่าตัวแปรด้วยตนเอง หลังจากนั้นแต่ละกลุ่มนำผลการทดลองมาเปรียบเทียบกัน ในห้องเรียนปฏิบัติการสืบเสาะแบบ learning cycle นี้ ครูผู้สอนจะทำหน้าที่ให้ความช่วยเหลือคอยชี้แนะ เพื่อให้นักศึกษาทำการสืบเสาะและสร้างความรู้ได้เองอย่างมีประสิทธิภาพ

ในการศึกษานี้ได้ประเมินประสิทธิภาพของรูปแบบการเรียนการสอนที่พัฒนาขึ้นต่อการเรียนรู้ของนักศึกษาทั้งในด้านความเข้าใจในเนื้อหาและทัศนคติต่อการเรียน โดยในการประเมินนี้ได้ใช้เครื่องมือหลากหลาย ได้แก่ แบบทดสอบความเข้าใจในเนื้อหา, แบบสอบถาม, การสัมภาษณ์, การสังเกตการณ์ในห้องเรียน และการวิเคราะห์ผลงานของนักศึกษา

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CHAPTER I
INTRODUCTION

Overview

This chapter provides background of the research area for this thesis and demonstrates the purpose as well as the significance of this research project.

1.1 Background and Justification for Study

Life science is one of the disciplines taught in higher education. Of all life science curricula, biochemistry is a fundamental subject that integrates chemistry and biology to explain the chemical processes in living organism. Regarding the biochemistry courses, enzyme kinetics is of great significant topic (Kraut et al., 2003) because the enzymes are biological catalysts responsible for supporting almost all of the chemical reactions that maintain life process (Metzler et al., 2001, p.455). It is thus essential for students to understand enzyme activity in order to understand how life can exit. Understanding kinetics of the enzymes may also provide students the basic knowledge used to interpret the role of particular enzymes in metabolic control. Moreover, the knowledge of enzyme kinetics has found application in the pharmaceutical, food and beverage industries that students in life science may concern after graduation (Leach, 2002; Sime, 1999).

Many science education research works reveal students have difficulties in learning and understanding the enzyme kinetics concepts (Runge, Hill & Moran, 2006). The students encounter great difficulties in apprehending the reaction rate concepts. They do not know how data about the rate of the enzyme catalyzed reaction can be collected (Leach, 2002). Many students experience difficulties in understanding the relationship between the concentration of enzyme or substrate and the rate of reaction (Waelbroeck, 2006). They also are unclear of what the optimum pH of enzyme is or the effect of changing pH has on the enzyme kinetics constant (Garrattet

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al., 2000). Likewise, students can seldom give an appropriate reason to explain the effect of temperature on the rate of enzyme catalyzed reaction (Driel, 2002). In addition they seem to think that the Micahelis-Menten model is a perfect description of the kinetics of enzymes, rather than a model of the kinetics of some enzymes under defined conditions (Leach, 2002).

In science education, the laboratory exercise is one of the strategies that has been used to promote a better students' understanding of the scientific concepts (Cameselle et al., 2000). The laboratory exercise has been promoted as serving a wide variety of educational functions (Hofstein & Lunetta, 2004): developing and restructuring knowledge schemes, reflecting science as it is practiced by scientists, and developing skills in the process of science (Hofstein & Lunetta, 1982; Tobin, 1990). Therefore, many enzyme kinetics laboratories have been designed to demonstrate the pattern of the kinetics of enzymes and reinforce the concepts students learned in lectures (Boyce, Casey & Walsh, 2004). The classical enzyme kinetic laboratories may focus on measuring of rate reaction and the effect of enzyme and substrate concentrations, temperature, pH, and inhibitors upon enzyme activity (Hardee, Montgomery & Jones, 2000; Vullo, 2003). These laboratories are usually carried out using a single-beam spectrophotometer and require the use of highly purified enzyme and substrate reagents, which are usually very expensive. Economical enzymes kinetics laboratories which do not use special equipment are needed for student laboratories, particular in developing countries (Vicario, Casati & Iglesias, 1997). Regarding the 1999 National Education Act that serves as a basis for the educational reform of Thailand including higher education, one strategy and road map of higher education emphasizes on using knowledge-based economy and society and increasing capacity for self-dependence while at the same time decreasing dependence on foreign technology (Ministry of Education [MOE], 2004a, 2005; Office of the National Education Commission [ONEC], 2003). Therefore, the enzyme kinetics experiment that uses local materials is needed for Thai undergraduate science laboratories.

Science researchers have reported that seaweeds which are widely found in many parts of the world including Thailand contain bromoperoxidases (Kongkiattikajorn & Pongdam, 2006; Kongkiattikajorn & Ruenwongsa, 2006). The

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bromoperoxidases belong to a class of peroxidatic enzymes called the haloperoxidases. These enzymes require H_2O_2 and a halide ion to transform the third organic substrate peroxidatically accompanied generally by the latter's product having covalently-bonded halogen atom(s) (Littlechild, 1999). The bromoperoxidase from seaweeds mostly contain vanadium at the active site (Kongkiattikajorn, Ruenwongsa & Panijpan, 2006; Suthiphongchai et al., 2008). The vanadium bromoperoxidases are stable at high temperatures and in organic solvents, and resists high concentrations of hydrogen peroxide (Butler & Carter-Franklin, 2004). The haloperoxidases are of interest for basic and applied bioscientists because of their catalyzing a variety of substrates and increasing in pharmaceutical industry and environmental cleanup (Blunt et al., 2006; Thomas & Raja, 2005). Thus, bromoperoxidase from seaweeds has become increasingly attractive, and thought to be useful to impart basic biochemical knowledge of the catalytic mechanism of the enzymes. This is not only economical and suitable for undergraduate science courses with limited budgets, but also increases the relevance of teaching by making use of locally available materials for student laboratories.

In the assay of bromoperoxidase activity, monochlorodimidone (MCD) is commonly used as substrate for the enzyme (Tanaka et al., 2002). This assay reaction produces the colorless halogenated product that can be detected with difficulty since it requires a special instrument as UV-spectrophotometer (Jenwithisuk, 1996). Although there is a simple visual assay by brominating phenol red to bromophenol blue, this reaction produces three-intermediates and shows long lag time (Jenwithisuk, 1996). It has been suggested by Boonsiri (1989) that bromophenol red may be a better substrate for bromoperoxidase since it has less vacant site for bromination than that of phenol red. The bromophenol red is a good substrate in terms of not only being easily detected by observing the color change either visually or using a simple spectrophotomometer, but it also produces less intermediates. In addition, the reaction time to get final brominated product is shorter (Jenwithisuk, 1996). This study therefore chose bromophenol red as a substrate for bromoperoxidase. Thus, the condition for assay bromoperoxidase activity using the bromophenol red is needed to be optimized before being used in student laboratories.

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Many science education researchers suggest that providing students with hands-on laboratory experiences is not by itself enough (Abraham, 1998). The laboratory experiment in which students perform by following the given procedures to collect the expected results although may teach students some laboratory techniques and skills (Hart et al., 2000) or serve as visual aids for concepts already studied (Millar, 1998). However, most students just work as technicians without thinking (Hofstein & Walberg, 1995). This way of learning is claimed to be ineffective in developing students' understanding scientific concepts (Wallace et al., 2003). It also misleads students as to how scientists develop scientific knowledge (McGuness, Roth & Gilmer, 2002). Many science educators believe that it is a waste of time to perform the laboratory by following the laboratory manual without thinking (Boud et al., 1986; Hart et al., 2000).

Tobin (1990) suggested that meaningful learning is possible in the laboratory if students are given opportunities to manipulate equipment and materials in order to be able to construct their knowledge of phenomena and related scientific concepts. Gunstone and Champagne (1990) suggested that meaningful learning in the laboratory would occur if students were given ample time and opportunity for interaction. This situation needs to involve inquiry-based learning (Hofstein, Navon, Kipnis & Mamlok-Naaman, 2005). Of all the inquiry-based learning approaches, the learning cycle is one of the foremost in teaching and learning of science. Many science education researchers (e.g. Abraham, 1998) suggest that teaching and learning of science should utilize instructional materials that have some of the key characteristics of the learning cycle approach.

The learning cycle is a three-phase inquiry approach consisting of exploration, concept introduction, and concept application (Lawson, 2000). In the *exploration* phase, students are given experience with the concept to be developed through the laboratory activity. In the *concept development* phase, the students and/or teacher derives the concept from data, this usually being carried out during a whole-class discussion. The *concept application* phase gives the students the opportunity to explore the usefulness and application of the concepts. Many education researchers say that the learning cycle is an effective instructional strategy possessing many

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advantages. It has been found to be very effective for teaching science concepts, improving reasoning skills in students from first grade to college (Songer & Ho, 2005; Karplus, 1977, 1980; Lawson, 2001), and helping students eliminate scientific misconceptions (Lawson, 1986). It also can improve students' attitude toward science and science learning, and creates students' superior process skills (Abraham, 1998; Ates, 2005; Karplus, 1980). This approach provides benefits not only for students, but also for teachers. Teachers who use the learning cycle approach have reported that they focus more on higher-order and open-ended questions rather than fact-oriented questions, they are more open-minded and ask more high-level questions, and end up with students possessing superior science process achievement. In addition, it seems these teachers themselves have better attitudes towards science than the teachers who have not used the learning cycle approach (Abraham, 1998).

The learning cycle, as mentioned above, provides the outcomes that match the needs of higher education in Thailand: motivating students to become lifelong self-learners, providing them with skills and depth of content in their chosen career path, engaging them to develop good work habits and management skills as well as the ability to cooperate with others, and preparing them for the 'real world after graduation (MOE, 2003, 2004ab, Piyanuwat & Sugiva, 2000). Therefore, the learning cycle approach seems to be appropriate for Thai undergraduate science classes.

This research work aimed to enhance university students a better insight into enzyme kinetics. An effective and appropriate instructional activity has been developed for undergraduate laboratory. This instructional model is designed based on integration of the newly developed enzyme kinetics laboratory using bromoperoxidase from Thai local seaweed with the learning cycle approach.

1.2 Problem Statement

Teacher-centered approaches have been norm in teaching and learning of enzyme kinetics at university levels in Thailand as well as in many countries (Corneley et al., 1999; Justi, 2003). The enzyme-based laboratories are usually coordinated with lectures to give a better understanding of the practical aspects of the discipline (Boyce et al., 2004). However, these laboratories are costly due to cost of

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highly purified enzymes and a special instrument used for detection of the enzyme activity (Vicario et al., 1997). There is a need to develop an appropriate effective exemplary model of teaching/learning using local indigenous materials for students' enzyme laboratories.

Unfortunately, the traditional lecture-laboratory method fails to motivate the meaningful intellectual engagement that is the central mission and hallmark of the university experience because the traditional cookbook laboratory exercises are boring to many students and the students still fail to make the desired connections between the laboratory and the key concepts (Russell & French, 2002).

On the other hands, the learning cycle approach has been proved very effective in promoting students' understanding of scientific concepts (Gabel, 2003; Mitchell, 1992). However, none of the previous research studies had examined the effects of this approach on teaching enzyme kinetics which is regarded as the difficult topic for undergraduate science students. Additional research is thus needed to see if this approach is useful for such learning (Abraham, 1998).

1.3 Objectives and Research Questions

This research study has three principal objectives:

1. to develop a simple detection for bromoperoxidase activity using a new substrate, bromophenol red,
2. to develop hands-on experiment using Thai local seaweeds for students' enzyme kinetics laboratory, and
3. to integrate the concepts of the learning cycle to the newly developed hands-on activities as part of the Thai tertiary science classroom experience.

This research will address the following two questions.

1. Can the newly developed enzyme laboratory based on the learning cycle approach be used to enhance Thai university students' understanding of enzyme kinetics?
2. How do Thai university students perceive the learning cycle approach based on their experience of enzyme laboratory activity?

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1.4 Significance of the Study

The learning cycle used as an intervention in this work is based on experiments using bromoperoxidase from seaweeds found around the Gulf of Siam, which can be appropriately used as an alternative model to effectively promote student understanding of enzyme kinetics. This is not only economical and suitable for undergraduate science courses with limited budgets, but also increases the relevance of teaching by making use of locally available materials for student laboratories.

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CHAPTER II
LITERATURE REVIEW

Overview

This chapter presents a review of relevant literature for this research. It is divided into two parts: science and education. The scientific part begins with the background information of bromoproxidase and haloperoxidase that includes the structure and function of the enzymes and their sources especially in marine algae. This is followed by their role in living organisms and their application. Then the method for assay the haloperoxidases are described.

The educational part begins with the theoretical basis of constructivism and its view of learning and teaching science. This is followed by the learning cycle approach which is based on constructivist theory. A description of its theoretical framework, its characteristics and the use of this approach in general laboratory class are also provided. The details of the laboratory work in science education, their benefits, the laboratory work in teaching and learning of science, and inquiry-based laboratory are next. The meaning of attitudes, their impact in science education, student perceptions toward science learning and practical work, and epistemological belief, are described. This is followed by general information of enzyme kinetics in higher education: tertiary student conceptual understandings of enzyme kinetics, teaching and learning of this topic, and the enzyme kinetics experiments focussing at higher level.

At the end of this chapter, the summary of the literature review is also provided.

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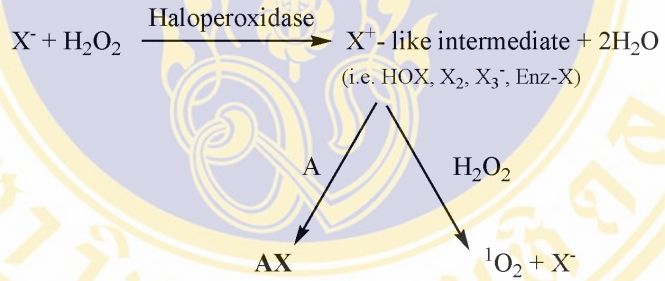
2.1 Scientific Review

2.1.1 Bromoperoxidase and Haloperoxidase

The bromoperoxidase belongs to a class of peroxidatic enzymes called the haloperoxidases. The haloperoxidases have a broad substrate range. Over the years, these enzymes have been shown to catalyze the halogenation of a variety of natural substrates to give products that are potential drugs and environmental agents e.g. β-ketoacids, cyclic β-diketones, steroids, substituted phenols, alkenes, alkynes, α-β-unsaturated acids and heterocycles (Niedleman & Geigert, 1986).

The haloperoxidases are able to catalyze halogenation reactions. These enzymes require both H_2O_2 (or an organic peroxide) and a halide ion to transform the third substrate (organic or inorganic) peroxidatically accompanied generally by the latter's product having covalently-bonded halogen atom(s) as shown in the following general equation:



A = substrate

X^- = halide ion (Br^-, Cl^- or I^-)

AX = halogenated product

The specific name of bromo- or chloro- or iodoperoxidase is given to an enzyme based on its capacity to use the halide (X^-) of the lowest atomic number and those of higher number(s). Thus chloperoxidases use chloride, bromide and iodide, the bromoperoxidases use bromide and iodide, and iodoperoxidases use only iodide (Niedleman & Geigert, 1986). A summary of some well-characterized haloperoxidases, grouped according to their ability to use halide ions, is presented in Table 2-1.

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Table 2-1 Some well-characterized haloperoxidases and their sources (Niedleman & Geigert, 1986)

Type of Haloperoxidases	Name
Bromoperoxidase	Lactoperoxidase (LPO) from milk, saliva and tears
	Overperoxidase from sea urchin egg
	Bromoperoxidase (BPO) from marine algae
	Bromoperoxidase (BPO) from bacteria *Pseudomonas aureofaciens* and *Streptomyces phaceochromogenes*
Chloroperoxidase	Myeloperoxidase (MPO) from human neutophils
	Eosinophils peroxidase (EPO) from human eosinophils
	Chloroperoxidase (CPO) from fungi *Dematiaceous hyphomycetes*
Iodoperoxidase	Thyroid peroxidase (TPO) from thyroid gland
	Horseradish peroxidase (HRPO) from horseradish root

It is now known that there are three distinct classes of the haloperoxidase. One of these consists of enzymes without a prosthetic group and as such have been detected in a number of bacteria (Bantleon, Altenbuchner & Van Pée, 1994). The remaining two classes are the so-called heme-containing haloperoxidase – exemplified by the CPO from the fungus *Caldariomyces fumago* (Hager, Morris, Brown & Erberwein, 1966) or MPO from white blood cells (Niedleman & Geigert, 1986) – and the vanadium-containing haloperoxidase that binds a vanadate ion as a prosthetic group. The example enzymes within the class of vanadium-haloperoxidase are vanadium bromoperoxidases, which are isolated mainly from many species of marine algae (Garcia-Rodriguez, Oshiro, Aibara, Izumi & Littlechild, 2005; Soedjak, Everett & Butler, 1991), and vanadium chloroperoxidases, which are isolated from certain terrestrial fungi (Butler, 1998). Enzymes representing these three classes not only differ in the nature of their prosthetic group but also in at least two other aspects: catalytic mechanism and stability, and these are described below.

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In the metal-free haloperoxidases, a specific halide-binding site appears to be absent in these enzymes and hydrophobic organic compounds may fit into the active-site pocket for halogenation at preferential sites (Littlechild, 1999).

The heme-containing haloperoxidases have protoporphyrin IX. Thus the heme prosthetic group at the active site of the enzyme has been shown to have a dual role in the catalytic function of haloperoxidases: (1) the metal (i.e., iron (III)) activates the heterolytic cleavage of the O–O peroxide bond and stores one oxidizing equivalent, and (2) the porphyrin regulates the oxidation-reduction potential and stores one oxidizing equivalent. The reaction thus can occur by changing the oxidation state of the metal to be active intermediate. This intermediate then transfers electron to halides or organic compounds to return itself to original state (Figure 2-1) (Chen & van Pée, 2008; Littlechild, 1999).

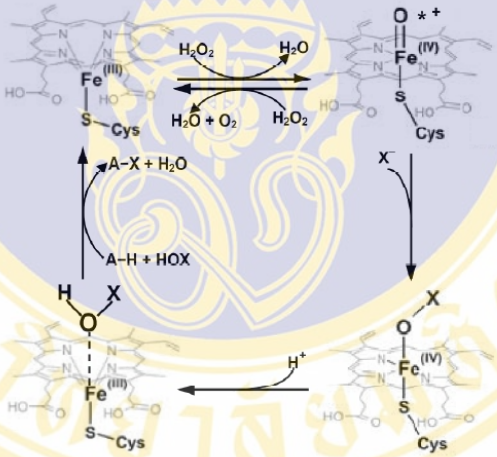

Figure 2-1 Reaction mechanism of heme-haloperoxidases in halogenation (Chen & van Pée, 2008) A–H, organic substrate; X = Cl^-, Br^-, or I^-

In vanadium-containing haloperoxidases, the transition metal does not change its redox state (Figure 2-2) as the iron in heme-containing haloperoxidases (Herrika, Renirie, Dekker, Barnett & Wever, 1997). The amino acid residues that participate in vanadium binding site provide rigid oxyanion-binding made up of an intricate hydrogen bond network between the amino acid residues and vanadate. The histidine side chain which does not interact directly with the vanadium cofactor has been

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proposed to participate as a proton donor and acceptor during the enzymatic reaction (Figure 2-3) (Butler & Carter-Franklin, 2004; Ligtenbarg, Hage & Feringa, 2003).

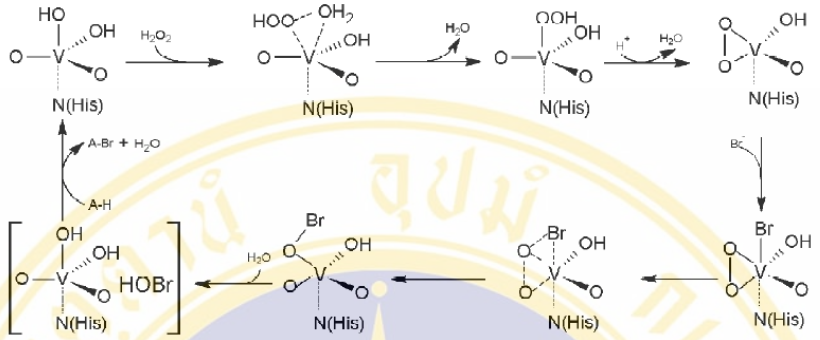

Figure 2-2 Basic reaction mechanism of vanadium-haloperoxidases in halogenation (Chen & van Pée, 2008) A–H, organic substrate

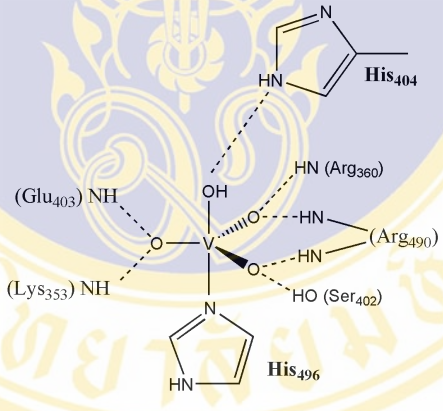

Figure 2-3 Proposed coordination of the vanadium active site in vanadium haloperoxidase (Ligtenbarg et al., 2003)

Vanadium-containing haloperoxidases not only possess a very high stability but also resist high concentrations of H_2O_2 and the product (HOX) that would readily inactivate the heme-containing haloperoxidases (Hemrika et al., 1997). Of all haloperoxidases, bromoperoxidases are predominated in nature (Murphy, 2003) especially in marine algae (Carter-Franklin, Parrish, Tschirret-Guth, Little & Butler, 2003).

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2.1.2 Marine Algae Containing Bromoperoxidase

Bromoperoxidase was firstly identified from marine red algae *Bonnemaisonia hamifera* by Theler, Cook, Hager and Siuda in 1978 (Neidleman & Geigert, 1986). Since that time, additional bromoperoxidases have been discovered in red, green, and brown marine algae by many researchers. Some well-characterized algae containing bromoperoxidase are presented in Table 2-2.

Table 2-2 Some well-characterized marine algae containing bromoperoxidases

Marine algae containing bromoperoxidases	
Rhodophyta (Red)	
Amphiroa hancockii	*Galaxaura squalida*
Asparagopsis taxiformiis	*Gracilaria sp.*
Bonnemaisonia hamifera	*Haloplegma duperreyi*
Brypthamnion triquetrum	*Kappphycus alvarezii*[a]
Chondria atropurpurea	*Laurencia chondroides*
Chondria enicophylla	*Laurencia intricate*
Coelothrix irregularis	*Laurencia lata*
Corallina officinalis[b]	*Laurencia obtuse*
Corallina pilulifera[c]	*Laurencia papillosa*
Dasya pedicellata	*Laurencia poitei*
Dasya sp.	*Liagora sp.*
Euchewna isiforme	*Ochrodes secundiramea*
Eucheuma serra[a]	*Solieria robusta*[a]
Galaxaura comans	
Chlorophyta (Green)	*Halimeda opuntia*
Bryopsis pennata	*Halimeda tuna*
Caulerpa raccemosa	*Penicillus capitatus*
Chaetomorpha linum	*Penicillus dumentosus*
Cladophorales sp.	*Penicillus lamourousii*
Caldophoropsis memvranacea	*Rhipocephalus phoenix*
Codium isthmocladum	*Udotea flavellum*
Codium repens	*Udotea wilsoni*
Dictyosphaeria cavernosa	*Ulva lactuca*
Halimeda discoidae	*Valonia macrophysa*
Halimada incrassate	
Halimeda foreauii	
Phaeophyta (brown)	
Ascophyllum nodosum	
Dictyota divaricata	
Laminaria saccharina[a]	
Lobophora variegata	

Data from Neidleman & Geigert (1986)
[a]Data from Kamenarsk, Taniguchi, Ohsawa, Hiroka & Itoh (2007)
[b]Data from Carter, Beatty, Simpson & Butler (2002)
[c]Data from Itoh, Izumi & Yamada (1987)

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In Thailand, the bromoperoxidase activity has been identified in many species of *Gracilaria* red algae found around the Gulf of Siam, for examples, *G. tenuistipitata* from Lam Sok and Pattani (Kongkiattikajorn & Ruenwongsa, 2006; Suthiphongchai, Boonsiri & Panijpan, 2008), *G. fisheri* from Kohyor and Pattani (Kongkiattikajorn & Pongdam, 2006; Suthiphongchai et al., 2008), *G. changii* from Ao Lane and Lam Sok, *G. edulis* from Ao Lane, *G. firma* and *G. salicornia* from Lam Tian (Suithiponchai et al., 2008).

After the discovery of various bromoperoxidases in many kinds of marine algae, considerable research effort has been made to understand the function of these enzymes in the living organisms.

2.1.3 Roles of Bromoperoxidase and Other Haloperoxidases in Living Organisms

For over 30 years, haloperoxidases were the only halogenating enzymes that had been identified and it was largely accepted that these enzymes were responsible for almost all biosynthesis of halometabolites (Butler & Carter-Franklin, 2004; Murphy, 2003). There are some evidences supporting the involvement of halopereoxidases in the production of some natural organohalogens, for examples, bromoform biosynthesis in the algae *Bonnemaisonia hamifera* and *Penicillus capitatus* appears to involve the action of a bromoperoxidase on 3-oxooctanoic acid (Theiler, Cook & Hager, 1978), and thyroid peroxidase has been shown to catalyze the iodination and coupling of tyrosine to yield the hormone thyroxine (Dème, Fimiani, Pommier & Nunez, 1975).

Many reports show that haloperoxidases in mammals namely MPO, LPO and EPO demonstrate both antimicrobial (Albrich, McCarthy & Hurst, 1981; Jong, Henderson & Klebanoff, 1980; Thomas, Bates & Jefferson, 1981) and cytotoxic activities (Clark & Klebanoff, 1977; Everse & Coates, 2004; Jong & Klebanoff, 1980). In the presence of hydrogen peroxide and halide ions (X^-), these enzymes produce hypohalous acids (HOX), reactive substances, which are responsible for toxicity effects to target cells (Albrich et al., 1981; Thomas et al., 1981).

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Currently, over 4,500 halogenated natural products (halometabolites) are known to be produced by haloeproxidases (Gribble, 2004). These products play amazing variety of structures and are synthesized by a diverse range of organisms in which many play important biochemical and physiological roles (van Pée, 1996) for examples, the lone star tick uses 2,6-chlorophenol as a sex-phenromone (Harper, 2000), while 4-chloroindolyl-3-acetic acid is a plant growth hormone (Harper, 2000; van Pée, 1996). Several halometabolites, particularly those of marine origin, appear to have a defensive role (Blunt, Copp, Munro, Northcote & Prinsep, 2006), and some are medically valuable and include antibiotics (e.g. chlortetracycline and vancomycin), antitumor agents (e.g. rebeccamycin and calicheamycin), and anti-HIV agents (e.g. chloropeptin I, ambigol A) (Chen & van Pée, 2008).

2.1.4 Application of Bromoperoxidases and Other Haloperoxidases

Bromoperoxidases as well as other haloperoxidases are of interest for basic and applied bioscientists because of their increasing importance in pharmaceuticals and environmental cleanups. The potential applications of these enzymes are described below.

2.1.4.1 As a Synthetic Tool

Haloperoxidases have been used in halogenation which is a key step in the production of many industrial chemicals. For example, haloperoxidase is used to convert propylene to propylene oxide (Borole & Davison, 2007) to be used in production of other industrial chemicals such as polyurethane plastics, flame retardants, lubricants, oil field drilling chemicals and textile surfactants (Dow Chemical Company, 2008).

2.1.4.2 As a Pharmaceutical Agent

Regarding the antibacterial properties of hypohalous acid, haloperoxidases have been used as therapeutic agent, for example, LPO in combination with thiocyanate ion and urea peroxide, as well as MPO in combination with iodide ion have been formulated with powdered drug preparations to preserve the drug from

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bacterial decomposition (Bjorck, 1982, Hasegawa & Kobayashi, 1983). The enzymes also have been used to preserve milk from bacterial growth (Montgomery, 1986; Nakada, Dosako, Hirano, Oooka & Nakajima, 1996).

By coupling the enzymes with antibody, the haloperoxidases could release their potent oxidant, hypohalous acid, and thus selectively destroy the invading agent or cell (Jong & Klebanoff, 1980). These enzymes have been used in toothpaste, mouthwashed, and chewing gum to kill oral bacteria (Kessler & Rosenbaum, 1984).

2.1.4.3 In Pollution Control

Haloperoxidases are importance in environmental clean up. These enzymes produce hypohalous acid which acts as biocide to inhibit slime growth that cause the fouling or plugging of pipe and filter which is a major problem in processing of water from the pulp and paper mill as well as other industries (Orndoff, 1983). The halopeoxidase namely horseradish peroxidase is capable of removing carcinogenic aromatic amine and phenol from coal conversion waste water (Klibanov & Morris, 1981; Klibanov, Tu & Scott, 1983).

2.1.4.4 For Analytical Diagnostics

Radioisotope such as ^{125}I and ^{131}I are used to label proteins in order to study biological process of that proteins (Manthey, Hager & McElvany, 1984; Tuszynski, Knight & Srivastava, 1983). Haloperoxidase provides a mean by which the radioisotopes are rapidly, mildly and stably attached to the protein, yielding high specific radioactivity with retention of the protein's biological activity.

Due to the ability to utilize different halide ions, the haloperoxidases have been used as a selective probe for measuring the level of a particular halide ion in a mixture of halides, for example, the use of IPO in specific measuring of iodide in the presence of bromide, chloride and fluoride (Grim & Lockhart, 1979).

By coupling enzyme assays, the haloperoxidase has been used in quantitative analysis of glucose in the blood by measuring the amount the amount of H_2O_2 produced in the present of glucose oxidase (Tang, 1992).

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The haloperoxidases have also been used in immunoassays. The enzymes were used to measure the presence of antigen/antibody complex in which ether species was labelled with the enzymes (Fonong & Rechnitz, 1984).

2.1.5 Method for Assay Haloperoxidases

The classical assay for haloperoxidases employs a synthetic substrate, monochlorodimedone (MCD). It results in the halogenation of MCD (Fiugure 2-4) which can be followed at 290 nm (Colin et al., 2003; Van Peé & Lingens, 1985).

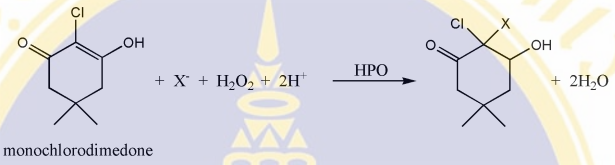

Figure 2-4 The haloperoxidase-mediated halogenation and oxidation reaction using MCD as substate, X = Br or Cl

The haloperoxidase activity could also be assayed by modification of tyrosine either as free tyrosine (Figure 2-5a) or as tyrosyl residues in protein (Figure 2-5b). The reactions can be followed at 310 nm and 279 nm respectively (Jervis, Jervis & Giovannelli, 2005).

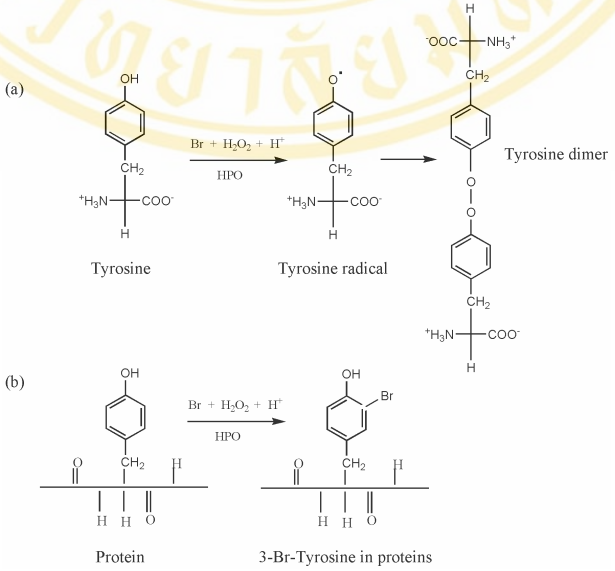

Figure 2-5 The haloperoxidase-mediated halogenation and oxidation reaction by modification of tyrosine

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The enzyme assay by using MCD as substrate or by modification of tyrosine produces the colorless products, these reactions thus require a special instrument as UV-spectrophotometer for detection. Other substrates, tartrazine and lissamine yellow, in which their reactions could be followed using a simple spectrophotometer have been suggests to be used for assay the haloperoxidase activity (Jervis et al., 2005).

In the enzyme assay using tartrazine as substrate, oxidation proceeds in two one-electron steps producing a tartrazine radical that then undergoes a series of further spontaneous reactions to low molecular mass products (Figure 2-6). The overall reaction results in bleaching of the tartrazine, a reaction followed readily at 428 nm. A similar reaction occurs with lissamine yellow with the reaction being followed at 403 nm (Jervis et al., 2005).

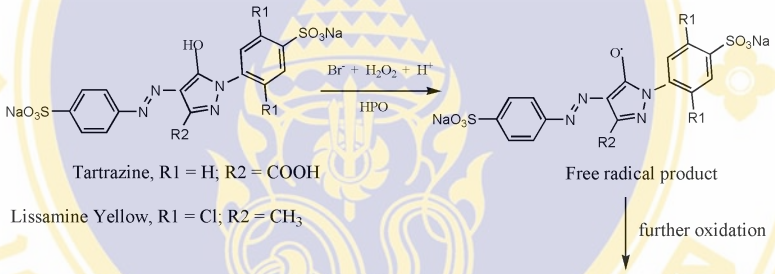

Figure 2-6 The haloperoxidase-mediated halogenation and oxidation reaction using Tartrazine or Lissamine Yellow as substrate

Another substrate, phenol red (PR) has been reported to be used widely at present for assay haloperoxidase activity (Jervis et al., 2005). Phenol red (PR) is a one of the well-known dyes of the sulfonphthalein family (the phenol reds) used as common pH indicators. It is structurally related to the other two indicators, the dibrominated phenol red (Br_2PR) called bromophenol red, and the tetrabrominated phenol red (Br_4PR) called bromophenol blue (Figure 2-7). In a stepwise bromoperoxidatic bromination of PR to Br_4PR the intermediates are expected to be Br_1PR, Br_2PR and Br_3PR. These indicators have different pKa's, in other words, distinctly different colors at the same pH over a certain range. Thus in the presence of the haloperoxidase enzyme and the substrates, H_2O_2, Br^- and Br_2PR at appropriate pH,

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the color of Br_2PR changes to a deeper blue which can be followed visually and colorimetrically at 590 nm.

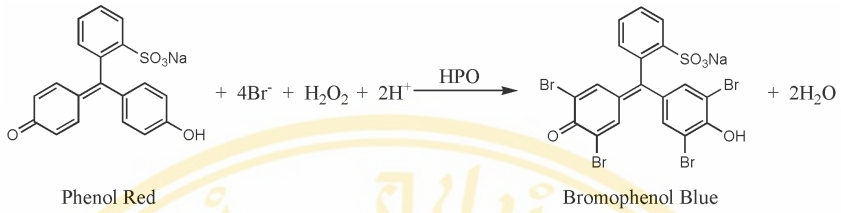

Figure 2-7 The haloperoxidase-mediated halogenation and oxidation reaction using phenol red as substrate

Although phenol red is a good substrate in terms of being easily detectable by observation the color change visually or measuring the increases in absorbance at 590 nm using a simple spectophothometer, this approach sometimes show long lag time (Jenwithisuk, 1996). It has been suggested (Boonsiri, 1989) that that bromophenol red can be used instead of PR because it has less vacant site for bromination (Figure 2-8). In a stepwise bromoperoxidatic bromination of bromophenol red to Br_4PR, the intermediates are expected to be Br_2PR and Br_3PR. Therefore the assay reaction using bromophenol red is two times more sensitive than PR.

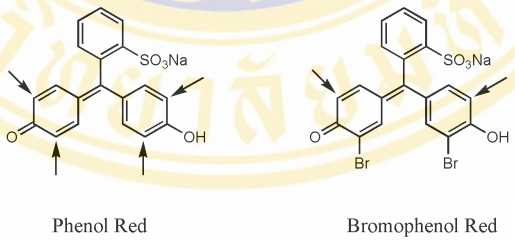

Figure 2-8 Proposed sites for bromination (arrow, →) of phenol red and bromophenol red

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2.2 Educational Review

2.2.1 Constructivism

2.2.1.1 Theoretical Basis of Constructivism

Constructivism has become increasingly accepted as a viable theory of knowledge (Roth, 1995) and comes to be an important theory in learning and inquiry (Bevevino, Dengel & Adams, 1999; Straits & Wilke, 2007). At the heart of constructivist view is the idea that knowledge about the world outside is viewed as human construction. Accordingly, learning is not viewed as transfer, but as active construction by an individual (Duit & Treagust, 1995, 1998). The main tenet of constructivist theory is that existing ideas which learners may hold are used to make sense of new experiences and new information (Appleton, 1997). Learning, therefore, occurs when there is a change in the learner's existing ideas, either by adding some new information or by organizing what is already known. In addition, there are social aspects of the construction process; although individuals have to construct their own meaning of a new idea, the process of constructing meaning always is embedded in a particular social setting of which the individual is part (Duit & Treagust, 1995, 1998).

According to constructivist theory, learning is actively constructed by an individual on the basis of the knowledge already held (Duit & Treagust, 1998). Learning in constructivism is viewed as conceptual development in much the same way as introduced by the work of Piaget (1954 cited in Duit & Treagust, 1998). A person's mental representations are produced during progressively more complex interactions by that individual with the world of physical objects. New information is initially assimilated by existing mental structures. If this assimilation proves inadequate – that is, the new information cannot be understood in terms of the existing mental structure, accommodation takes place – that is, a modified structure evolves. The interplay between assimilation and accommodation, known as equilibration, results in mental structures which are progressively more decentred – that is, are less and less concerned with the immediate, the concrete and the personal (Abraham, 1998; Bell, 2005). Piaget's work encouraged science educators to take an active view of

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learning and attempting to match the types of experiences given to children to the general pattern of cognitive growth – working with concrete objects for younger children and progressing to ideas requiring more formalization later (Duit & Treagust, 1998). In addition, from Piaget's perspective, some concepts such as energy were seen as too abstract for the majority of students to learn until they had reached to stage of formal operation since maturation is seen as one major influence on learning which requires time to develop. This idea has an impact on sequencing of concepts in science curriculum (Bell, 2005; Renner & Lawson, 1973).

However, the constructivist view of learning has involved a shift in focus away from viewing meaning-making in terms of cognitive processes in the individual, towards an account of individuals as they function in social contexts (Leach & Scott, 2003; Palincsar, 1998; Straits & Wilke, 2007). There has been a similar shift in focus in research into teaching and learning science (Solomon, 1994a). A social constructivist view of learning considers both the development of the individual construction of meaning toward the socially agreed to knowledge and the reconstruction and transformation of the culture and social knowledge itself (Bell & Gilbert, 1996; Palincsar, 1998). Knowledge and understandings according to social constructivist perspective are constructed when individuals engage socially in talk and activity about shared problems or tasks. Making meaning is thus a dialogic process involving individuals-in-conversation. A more experienced person of a culture can support a less experienced person by structuring tasks that making it possible for the less experienced person to perform them and to internalize the process – that is, to convert them in the mind (Driver, Asoko, Leach, Mortimer & Scott, 1994; Palincsar, 1998). Thus, the main goal of a social constructivist view of learning, thinking and the mind is to create an account of human mental process that recognize the essential relationships between mental processes and their social, cultural and institutional setting (Bell, 2005).

2.2.1.2 Constructivist View of Learning and Teaching Science

Constructivist views do stand in opposition to long-established majority traditions in science education. In that tradition the dominant image has been the

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transmission, from teacher and text to students, of single-track, logically organized knowledge (Hawkins, 1994). The core commitment of a constructivist position is that knowledge is actively built up by the learner, and the teacher acts as a facilitator of knowledge construction (Driver et al., 1994; Duit & Treagust, 1995; Straits & Wilke, 2007).

Constructivist learning is always an interpretative process involving individual's constructions of meaning relating to specific occurrences and phenomena. New constructions are built through their relation to prior knowledge – and the pedagogic challenge for teachers is to focus on students' learning with understanding, rather than the more common emphasis on covering content (Watts, 1994). To learn science from a constructivist theory implies direct experience with science as a process of knowledge generation in which prior knowledge is elaborated and changed on the basis of fresh meaning negotiated with peers and teacher since constructivist view that students make sense of what is presented to them by attempting to associate it with what they already know (Watt, 1994). To be truly learned, new information experiences by a student must first be lodged onto the cognitive foundations of what the person already understands. Once information is properly assimilated with previous understanding, a person can make predictions about outcomes and effectively explain information to others (Fosnot & Perry, 2005; Lord, 1997).

Constructivist teaching generally involves creating learning opportunities for students to make their own ideas explicit, share them with others, subject them to critical scrutiny and test their robustness by observation and/or experiment (Hodson, 1998). However, the teaching of science requires more than challenging learners' prior ideas through discrepant events. Teaching science involves the learner entering into a different way of thinking about and explaining the natural world; becoming socialized to a greater or lesser extent into the practices of the scientific community with its particular purposes, ways of seeing, and ways of supporting its knowledge claims (Driver et al., 1994). Before this can happen, individuals must engage in a process of personal construction and meaning making. Characterized in this way, learning science involves both personal and social processes (Driver et al., 1994).

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Teaching under constructivism can facilitate student's learning for understanding of science content in a much more appropriate way than traditional approaches (Duit & Treagust, 1995). The teacher acts as a facilitator of knowledge construction (that is a guide in students' individual construction processes) rather than as a person who transfers knowledge to the brain of students (Duit & Treagust, 1995; Fosnot, 2005). The teacher's role needs to adapt within classrooms to encourage more student-student interaction that in turn promotes negotiation to reach consensus about the meaning of scientifically acceptable concepts (Hand, Treagust & Vance, 1997). Matthews (1997) suggested that in the constructivist classroom, the teacher should help students learn how to learn, ensure equity for all students and create environment to support students' learning (such as providing resources to help students learn and creating a friendly). The teachers should challenge students to present their ideas, listen to them, and identify and nurture the scientific talent and interests of all students. In addition, the teacher should use student's ideas, experiences, and interests in teaching/learning.

Several models of learning and teaching in science based on constructivist theory have been proposed. Posner et al. (1982) described the conditions which determine whether cognitive change in learners will occur. That is learners must recognize the inadequacy of their existing conceptions (dissatisfaction), understand the new concepts being taught, and recognize them to be both plausible and fruitful for their own learning. These points serve as a useful clarification of learning and teaching situations but do not provide general indications as to what learners might do, and in turn, what teachers might do to facilitate learning (Appleton, 1997). In addition, Mayer (1983) suggested three conditions necessary for learning and teaching: (1) the learner must receive presented material; (2) the learner must have relevant prior knowledge or schema; and (3) the learner must activate that relevant prior knowledge. These models serve as examples of what has been available to teachers interested in applying constructivist ideas in the classroom. However, the models tend to be limited in scope and provide only a few clear indications for what a teacher might do to help students learn for understanding (Appleton, 1997).

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The learning cycle is one of the learning and teaching approach based on constructivism (Renner & Lawson, 1973). It has been demonstrated clearly on what learners and/or teachers might do in the classroom (Colburn & Clough, 1997; Lawson, 1986; Renner & Lawson, 1973). In this approach, opportunities for students to reveal their prior science idea exist in two ways: (1) students make predictions before exploring; and (2) students generate hypotheses to explain new phenomena (Blank, 2000). The learning cycle approach has been suggested by many science educators (e.g. Abraham, 1998) to be used in teaching and learning of science.

2.2.2 The Learning Cycle Approach in Science Education

2.2.2.1 Theoretical Framework of the Learning Cycle Approach

The learning cycle approach was an early inquiry-oriented science teaching strategy in research more than three decades ago (Abraham, 1997; Bevevino et al., 1999; Lawson, 1986). This approach is based on constructivist theory and was first developed by Robert Karplus following the major steps in the intellectual development proposed by Jean Piaget (Abraham, 1998; Karplus, 1977, Renner & Lawson, 1973).

Piaget suggested that cognitive development is a function of four processes (1964 cited in DeBoer, 1991). The first three are physiological development, personal interaction with one's environment, and social transmission or direct instruction. The fourth process Piaget called "self-regulation", the process by which an individual is first faced with and subsequently transforms new knowledge to fit into existing mental structures. Self-regulation is also described as a movement from a state of cognitive conflict or disequilibrium to one in which cognitive order and equilibrium is regained. Disequilibrium provides the energy by which reorganization of mental structures takes place. According to Piaget (1970 cited in Abraham, 1998), learning occurs spontaneously through the process of assimilation, accommodation, and reorganization. If new information fits the expected outcomes, then this information is assimilated into the current mental structure. If, however, new information does not fit or understand in terms of the existing mental structure, disequilibrium occurs and accommodation is needed. As a consequence of accommodation, alternative mental

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structures are selected or constructed, driven by disequilibrium, until a good match between expected and actual outcomes occurs to restore equilibrium.

The learning cycle approach involves a set of instructional steps linked to this notion of cognitive development that is exploration must precede concept invention and discovery (DeBoer, 1991; Renner & Lawson, 1973). So students need to explore and experiment in order to allow more time for new concepts and insights to interact with their own initial conceptions (Renner & Lawson, 1973). Factor of the experience helps students to build operational-structures which can ultimately lead them to think abstractly about the world around them. In other words, it is experience with the materials of the discipline that produces the person who can understand abstract content and it is not studying abstract content which produces students who can interact with the materials and invent abstract generalizations. Thus, the laboratory must precede the introduction of an abstract generalization (Renner & Lawson, 1973). Data from an experiment must be shared and discussed. This requires that the students carefully examine their present beliefs which will, according to the Piaget's model, develop and change structures since the social interaction can lead to conflict, debate, shared data, and the clear delineation and expression of ideas. The factor of social interaction is valuable in building and rebuilding structures (Renner & Lawson, 1973). However, data from experiment can be very threatening because it is too often disequilibrium. But to the sensitive, concerned science teacher, disequilibrium is an opportunity to introduce the student to the major conceptualizations of the discipline which will produce equilibrium (Renner & Lawson, 1973).

Therefore, the learning cycle approach takes advantage in learning by sequencing instructional activities to be compatible with Piaget's cognitive development. In order to facilitate assimilation, the activities in exploration phase expose the learner to a segment of the environment that demonstrates the information to be accommodated. The activities in the second phase help the learner to accommodate the information. Finally, in order to organize the accommodated information, activities in the last phase are developed to help the learner to see the relation between the new information and other previously learned information (Abraham, 1998).

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2.2.2.2 Characteristics of the Learning Cycle Approach

The key characteristic of the learning cycle approach is an inquiry-based teaching strategy that divides instruction into three phases. This approach combines experience with social transmission and encourages self-regulation (Karplus, 1977; Lawson, 2000; Renner & Lawson, 1973).

The three phases of the learning cycle are *exploration*, *concept introduction*, and *concept application* (Karplus, 1977; Lawson, 1986, 2001). During the *exploration* phase, students engage in laboratory experiments and collect data on observed science phenomena with minimal guidance. The exploration engages students in some level of disequilibrium or cognitive conflict as they struggle to make sense of their observations and experiences. In the *concept introduction phase*, the teacher guides students toward compilation or representation of their collected data. Through use of the data and classroom discourse, students prepare a statement that represents their understanding of the science concepts. After they have attained concept understanding, the teacher may provide related scientific terminology. In the third phase, *concept application*, students expand their understanding by using the concept in a different context.

The most important characteristic of the learning cycle approach is the presence of all phases in a specific sequence (Abraham, 1998; Marek, Askey & Abraham, 2000). The exploration phase comes first since the information exposed will be used inductively during the concept introduction phase. This is not to deny the role that prior knowledge and deductive thought play during exploration. Indeed, Lawson (1986) discusses various kinds of thinking processes actively during exploration. The new experience raises questions or complexities that students cannot resolve with their present conceptions or accustomed patterns of reasoning. In other words, it provides the opportunity for students to voice potentially conflicting and at least partially inadequate ideas that can spark debate and analysis of the reasons for their ideas. The data generated by the laboratory will be generalized to a concept. The exploration phase provides an excellent opportunity not only for students to become aware of their personal concepts about specific natural phenomena but also for teachers to assist students in questioning their understanding of the natural world as

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well as helping them with misconceptions they may uncover (Allard & Barman, 1994; Lawson, 1986). In addition, laboratory experiences are required in the first phase of the learning cycle in order to engage the student in a motivating activity (Allard & Barman, 1994). The use of laboratory activities followed by discussion is not only appropriate but also essential for concept development (Allard & Barman, 1994).

The learning cycle serves for direct teaching of concepts by the teacher – either by demonstration, lecture, reading, or other teacher-planned and -organized activities (Lawson, 1986). Thus, in the second phase, it favors the direct expository method suggested by Ausubel (DeBoer, 1991). This phase is preceded, however, by an exploratory phase in which the student becomes familiar in a first-hand way with the objects of learning. Thus, direct teaching of a concept is not attempted until the student has had a chance to explore the materials and develop some familiarity with them. In addition, in accordance with Piaget's theory, the student is expected to experience some cognitive conflicts, or disequilibrium, and the desire to achieve equilibrium in this phase. This provides the energy that makes the direct teaching of the concept successful. It should be pointed out that experiences in an exploration phase may raise questions to students and then they will discover the meaning of the concepts in their own mind (DeBoer, 1991). Although such independent discovery may occur, providing students' opportunity to use knowledge they had discovered also is one important feature needed for successful learning.

The third phase serves for a further strengthening of the concept through the application of the newly acquired material to new contexts. The concept application phase is necessary for some students to abstract the pattern from its concrete contexts and/or to generalize it to other situations. Without a number and variety of applications, the pattern may not be abstracted from contexts or its generality may remain restricted to context used during its definition (Lawson, 1986). This idea is in keeping with Ausubel's idea of networks of concepts that increase in meaningfulness as they appear in more and more new contexts (DeBoer, 1991).

Teaching and learning characterized by the learning cycle approach requires the teacher to be a facilitator of learning and not just a dispenser of knowledge (Allard

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& Barman, 1994). During exploration, the teacher acts as a facilitator, providing materials and directions, guiding the physical process of the experiment. After the exploration, the teacher promotes a discussion period in which students share their observations with classmates. This is the time in which the teacher connects student experiences to the target science concept including the identification of scientific vocabulary. Once the concept has been labelled, the teacher provides additional activities to give students opportunities to apply their recently formed understandings to new situations (Lawson, 1986; Settlage, 2000). It demands that the classroom atmosphere be one where students can explore and test different ideas (Allard & Barman, 1994). It requires enough time to identify and express students' pre-conceptions, examine their usefulness and apply the new concepts and ideas in a context familiar to them (Ates, 2005).

2.2.2.3 The Learning Cycle in General Laboratory Class

The learning cycle is a robust instructional approach benefiting from extensive research and moves science teaching and learning beyond simply a hands-on, discovery approach (Settlage, 2000).

The learning cycle approach was first used in a primary school science program called the Science Curriculum Improvement Study (SCIS) (Abraham, 1998; Lindgren & Bleicher, 2005). This approach has been well researched in comparison studies of SCIS programs and non-SCIS programs. The research findings are that students learning science through SCIS have better attitudes toward science, greater motivation toward learning science, higher self-concepts, and more positive attitudes toward inquiry. Further, SCIS teachers taught more science, were more open-minded, used higher order questions, and had students with superior science process achievement and better attitudes toward science than non-SCIS teachers (Abraham, 1998; Karplus, 1977; 1980).

As a result of the success of the SCIS program, many science educators consider the learning cycle to be a potentially useful model for instruction and curriculum development (Abraham, 1998). Therefore, many groups developed curricula using the learning cycle approach. Like the SCIS program, using the learning

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cycle in other curricula often improves more positive student attitudes toward sciences and science instruction relative to other more traditional approaches. This was true at varying levels from primary school to college (Abraham, 1998; Allard & Barman, 1994). Like SCIS program, learning cycle teachers spend more time in engaging in classroom activities with their students than non-learning cycle teachers (Marek & Methven, 1991).

In addition, improvements among students using the learning cycle approach in terms of process skill development, attitudes toward science and scientific inquiry (Bevevino et al., 1999; Marek & Methven, 1991), content learning attainment, and in reducing misconceptions were observed (Abraham, 1998; Lawson, 2001). The learning cycle approach can bring about growth in reasoning abilities greater than those of other instructional strategies (Lawson, 1986, 2001; Marek & Methven, 1991). Using learning cycle in the classroom can actively engage students in thinking, talking, reading, and writing about science (Gillis & MacDougall, 2007; Lawson, Drake, Johnson, Kwon & Scapane, 2000; Musheno & Lawson, 1999). The review of science education research seems to indicate that the learning cycle approach has real promise in terms of attitudes toward science, scientific inquiry and science instruction, content achievement and reasoning ability.

Since this time, names of phases have been modified and more phases have been added to the learning cycle model (Abraham, 1998), including the 4E (Friedrichsen, 2001) and 5E (Balci, Cakirouglu & Tekkaya, 2006) models. For example, the Biological Science Curriculum Study (BSCS) curriculum uses the phase names engage, explore, explain, elaborate, and evaluate. Lavoie (1999) modified the learning cycle by expansion phases and given students opportunities for hypothesis testing and/or designing the experiments to enhance process skills and logical thinking abilities. Eisencraft (2003) also suggested the addition of “elicitation” and “extension” phases (7E) incorporated in the 5E model. Some change the name of concept introduction phase to “clarification” to emphasise the learner’s primary role in knowledge construction (Abraham, 1998). Others renamed the learning cycle as “Oriented Learning Cycle” to emphasize the critical role of discourse and social interaction (Glasson & Lalik, 1993). Blank (2000) also revised the learning cycle and

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uses term “Metacognitive Learning Cycle” to emphasize formal opportunities for teachers and students to talk about their science ideas. In addition, a review of science education research stated refinements involving the combination of the learning cycle with other instructional techniques such as cooperative learning activities (Lavoie, 1999) and concept mapping activities (Odom & Kelly, 2001).

The learning cycle has been used in various subjects such as physics (e.g. Ates, 2005), mathematics (e.g. Simon, 1994), engineering (e.g. Svarovsky & Shaffer, 2007), and biology (e.g. Lauer, 2003; Lee, 2003). This approach has been found to be an effective method to help the concrete operational students understand abstract ideas (Gabel, 2003; Mitchell, 1992). Students in the learning cycle classrooms seem to gain a better understanding than those students in the traditional classrooms of scientific concepts such as direct current circuits (Ates, 2005), earth system (Assaraf & Orion, 2005; Kali, Orion & Eylon, 2003), genetics (Cavallo, 1996; Lawson, 1996), diffusion (Odom & Kelly, 1998, 2001), and osmosis (Lawson, 2000; Odom & Kelly, 1998, 2001). However, none of the previous research studies had examined the effect of this approach on teaching enzyme kinetics which is regarded as the difficult topic for undergraduate science students.

The use of learning cycle approach in general laboratory classes has proved very effective in promoting students’ understanding of scientific concepts. As mentioned earlier, in the learning cycle class, the laboratory experiences are viewed as an integral part of the lessons. These experiences are used as a vehicle to develop concepts or as a means to enhance or expand concept development (Allard & Barman, 1994).

2.2.3 Laboratory Work in Science Education

2.2.3.1 Laboratory Work in Science Education

The laboratory work has been given a central and distinctive role in the science curriculum, and science educators have suggested that rich benefits in learning accrue from using laboratory activities (Hofstein & Lunetta, 2004). The science laboratory has been promoted as serving a wide variety of educational functions. These functions

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include developing and restructuring knowledge schemes (Tobin, 1990), promoting inquiry and problem solving, reflecting science as it is practiced by scientists, and developing skills in the processes of science (Hofstein & Lunetta, 1982).

The science laboratory was a development of the latter third of the nineteenth century. It represented a major change in the way science was taught and a fundamental break from earlier book study of the science. Early laboratory work was justified primarily because of its ability to develop observational and inductive reasoning skills and because direct contact with the physical world was considered to be a unique characteristic of the science study. Science educators were so enthusiastic about the potential of the laboratory to develop the powers of the mind that the laboratory became the central focus of science instruction since their belief that the sciences, unlike many other disciplines, offered direct experience with natural phenomena of the physical world. Therefore, the laboratory work had to be the focus of science courses because the laboratory was the place where students could experience this contact with the physical world (DeBoer, 1991; Hofstein & Lunetta, 1982; Solomon, 1994b).

The laboratory later was to be used to solve problems – problems that were interesting and real to the students and problems that had social relevance. Along with this new idea for the laboratory came the development of the project method of teaching, which used a variety of instructional practices, including laboratory work, in the solution of real-world problems (DeBore, 1991). Under the project method, socially relevant problems, rather than the disciplines themselves, were to become the basis for organizing the curriculum. Any content that was learned would come from the solution of real-world problems. The science class was an ideal place to utilize the project method because available of the laboratory and the popularity of the project method in educational circles gave considerable support to the idea that the laboratory should be used for genuine inquiry activities (DeBore, 1991; Roth, 1994).

The progress in moving science laboratories in the direction of student problem solving had been slow because the laboratory periods for problem solving were time consuming. The laboratories were also cost consuming, required equipment and supplies, and took up space. Thus, teacher demonstrations were the greatest challenge

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to the laboratory since the teacher demonstrations were at least as effective as laboratory work in increasing student knowledge of science facts and principles. The laboratory demonstrations were needed to arouse interest in the subject and to make the subject clear; these helped students visualize the concept (DeBore, 1991).

Although the laboratory demonstration was a great time saver, many science educators attempted to use laboratory work in science courses since they believed that the laboratory work had more benefits than demonstration in providing student opportunities in doing science. This resulted in the costliness of laboratory work was to reduce the time spent in the laboratory to single-period labs. This enabled them to make a better connection between the lab and lecture room activities. With single-period lab, a laboratory period was just like any other period. Therefore it was always possible for the laboratory work to precede the text or lecture work and thus be used in the spirit of inductive problem solving that many preferred (DeBore, 1991; Nakhleh, Polles & Malina, 2002).

Laboratory work now has involved students' learning of concept and model of science as well as the development of their ability to be engaged in scientific inquiry (National Research Council [NRC], 1996, 2000). Laboratory work is used to represent idea of the world of things and to engage students in purposeful observations of and interventions into the world by using specially developed or commonly available objects and apparatus. Science educators believe that conceptual and procedural knowledge are intertwined for coping with laboratory situation and both are employed if the students are to be engaged in experiment activities (Hofstein & Lunetta, 2004; Psillos & Niedderer, 2002).

Since that time, many science education researchers have examined the value of laboratory on learning of science. Several attempts have been made to understand the effectiveness of the laboratory in terms of promoting students' understanding of the scientific concepts involved (Johnstone, 1997; Rollnick, Zwane, Stskun, Lotz & Green, 2001). Other work (Hart et al., 2000) casts doubt on the effectiveness of laboratory work to accomplish the aim of learning science content. Some researchers (Rollnick, Lubben, Lotz & Dlamini, 2002) have demonstrated an interest in using the

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laboratory work to develop students' understanding of the methods of scientific enquiry which is so-called procedural understanding.

2.2.3.2 Benefits of Laboratory Work in Science

The laboratory work has many benefits in science education. The laboratory not only provides experience with physical world, but also offers student opportunities to gain knowledge in the same way as scientists do (Nakhleh et al., 2002). In other words, laboratory appeals as way of allowing students learn with understanding and, at the same time engage in the process of constructing knowledge by doing science (Tobin, 1990). Science educators believe that the understanding of science is to be achieved in the first place not by reading about theories but by performing experiments and creating concepts at first-hand in the laboratory (Solomon, 1994b). In addition, science education literature articulates that laboratory work is an important medium for enhancing attitudes, stimulating interest and enjoyment, and motivating students to learn science (Hofstein & Lunetta, 2004).

Tamir (1991) gives five reasons to rationalize the use of laboratory in science: (1) science involves complex and abstract subject a matters, and, as a consequence, many students may fail to understand the concepts without the assistance and opportunities for carrying out activities in the laboratory; (2) students perceptions of actual investigations are an essential component of inquiry science learning, and laboratory work gives students opportunities to act like a real scientist; (3) laboratory experiences are essential for students to develop specific skills and scientific strategies; (4) laboratory work is important for identification, diagnosis and remediation of student alternative conceptions; and (5) laboratory work gives students enjoyable activities and they become interested in science.

2.2.3.3 Laboratory Work in Teaching and Learning of Science

Laboratory work is accepted by many science educators as a key part of teaching and learning of science at both secondary and tertiary levels (Nakhleh et al., 2002; Wellington, 1998). The laboratory in the science classroom has long been used to involve students in concrete experiences with objects and concepts (Hofstein &

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Lunetta, 1982). It is a unique educational setting in which students interact with materials and equipment and observe phenomena (Hofstein & Walberg, 1995). The science curriculum is infused with images of students conducting rigorous laboratory-based experiments, mimicking the behavior of real scientists in real scientific laboratories (McGuiness, Roth, & Gilmer, 2002).

The laboratory work used for teaching and learning of science has goals to encourage students' interest, to engage them with experiences of concepts, and to develop their practical science abilities (Hofstein & Lunetta, 1982, 2004; Nakhleh et al., 2002). The laboratory work is used to extend, illustrate and confirm accepted principles. In addition, there is a case for including opportunities for students to undertake their own investigations, in planning an experiment using their own initiative. The focus of such activities is not only the result obtained, but also the steps along the way: the design of experiment, the choice and use of the apparatus, the careful recording and interpretation of the results (Driver, 1986).

However, there is the mismatch between goals and reality, and the difference between school science and real science in using laboratory (Lunetta, 1998; McGuiness, et al., 2002). The tradition laboratory work is often seen by the teacher as a means of obtaining factual information and data from which conclusions will later be drawn (Hodson, 1998). It has usually been assumed that these data are pure and unaffected by students' existing ideas and, therefore, students have not usually been involved in the designing and planning of experimental investigation. There is also the problem that the laboratory work is physically separated from the lecture giving the theoretical principles and concepts it illustrates and the connection may not be evident (Boud, Dunn, & Hazel, 1986; Havdala & Ashkenazi, 2007). In addition, many laboratory classes follow a cookbook style (Berry, Gunstone, Loughran & Mulhall, 2001; McGuiness, et al., 2002) which emphasizes following specific procedures to collect data. Virtually no attention is given to the planning of the investigation or to interpreting the results (Tobin, Tippins & Gallard, 1994). Although this laboratory cookbook style can teach some laboratory techniques and skills (Hart et al., 2000) or serves as visual aids for concepts already studied (Millar, 1998), it gives students work as technicians who use low-level skills and abilities (Hofstein & Walberg, 1995). It

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has been criticized as placing very little emphasis on thinking (Raths, Wassermann, Jonas & Rothstein, 1986). Students may or may not learn something about the way scientists do things in such circumstances (Millar, Tiberghein & Maréchal, 2002). This approach is claimed to be not only an ineffective means of developing students understanding of science concepts (Gunstone & Champagne, 1990; Wallace et al., 2003), but also presents a misleading way of how scientists develop scientific knowledge (McGuiness et al., 2002). So, the laboratory fails to engage students in the thinking that precedes an experimental investigation that renders much of the ensuing laboratory work pedagogically useless (Hodson, 1998; Millar et al., 2002). There is much time wasted in a laboratory when students engage in data gathering without knowing the purposes for these tasks and without adequate opportunity for processing and analyzing (Boud et al., 1986; Hart et al., 2000).

However, many researchers believe that laboratory work may have real value. Tobin (1990) suggested that meaningful learning is possible in the laboratory if students were given opportunities to manipulate equipment and materials in order to be able to construct their knowledge of phenomena and related scientific concepts. Gunstone and Champagne (1990) suggested that meaningful learning in the laboratory would occur if students were given ample time and opportunity for interaction and reflection, however, this situation needs to involve inquiry-based learning (Hofstein et al., 2005; Kipnis & Hofstein, 2007).

2.2.3.4 Inquiry-based Science Laboratories

The inquiry-based laboratories have been suggested by many researchers to be potential to enhance students' meaningful learning, conceptual understanding, and understanding of the nature of science (Hofstein et al., 2005; Kipnis & Hofstein, 2007). Hofstein and Walberge (1995) suggest that inquiry-based laboratories are central to learning science, since students are involved in the process of conceiving problems and scientific questions, formulating hypotheses, designing experiments, gathering and analyzing data, and drawing conclusions about scientific problems or phenomena. Science education researchers articulates that inquiry-based experiences

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in the science laboratory are especially effective if conducted in the context of, and integrated with, the concept being taught (Kipnis & Hofstein, 2007).

The National Science Education Standards (NRC, 1996, 2000) reaffirm the conviction that inquiry is central to the achievement of scientific literacy. They suggest that science laboratories have the potential to be an important medium for introducing students to central conceptual and procedural knowledge and skill in science. Under the category of ability or skill, Bybee (2000) includes identifying and posing scientifically oriented questions, forming hypotheses, designing and conducting scientific investigations, formulating and revising scientific explanations, and communicating and defending scientific arguments. Many of these abilities and skills are in alignment with those that characterize inquiry-based laboratory work. Through development of inquiry skills, students are expected to gain an understanding of concepts and content, an understanding of the process of scientific inquiry, and an understanding of the nature of science, in addition to positive attitudes toward science (Abd-El-Khalick, Bell & Lederman, 1998; Brown, Abell, Demir & Schmidt, 2006).

The learning cycle approach is one of inquiry-based laboratories since a key aspect of the learning cycle approach is to attempt to engage students in meaningful inquires and with the aim of helping students construct meaningful concepts (Lawson, 2000; Wu & Tsai, 2005). Implementing the learning cycle may also mean eliminating the distinction between lecture and laboratory. The laboratory experiences in the learning cycle approach are designed to provide meaningful exploration at the beginning of the lessons. The lecture or information sharing occur as an outgrowth of the exploration activities, and other laboratory sessions provide opportunities for students to apply what they have learned in the previous phase to new situations (Allard & Barman, 1994).

Abraham (1998) has suggested that the science teachers should utilize instructional materials that have some of the key characteristics of the learning cycle approach. He has claimed that instructional strategy providing students with hands-on laboratory experience is not by itself enough, laboratory activities should be used to introduce concepts so that students are given the opportunity to construct knowledge from their own experiences and apply that knowledge to new situations. Especially,

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the science education research studies indicate the learning cycle approach is an effective method to help students understand scientific concepts, increase student's positive attitudes toward science (Ates, 2005; Cavallo & Laubach, 2001), motivate students to learn science, promote student's self-concepts, and improve positive attitudes toward inquiry (Abraham, 1998).

2.2.4 Attitudes toward Science Learning and Laboratory Work

2.2.4.1 Attitudes in Science Education

The development of positive attitudes toward science learning and laboratory work is a basic goal of science instruction (Fouts & Myers, 1992; Lavoie, 1999). This is because attitudes influence behavior and cognitive learning; positive attitude is often related to achievement (Crawley & Coe, 1990; Neathery, 1997; Shringley, 1990; White, 1998).

A problem that has been raised by those studying attitudes is the definition of *attitude* itself. There seems to be many concepts that relate to attitudes that may or may not be included in their definition; for example, feelings, beliefs, motivation, enjoyment, affects, self-esteem, and so forth (Kind, Jones & Barmby, 2007; Hasan, 1985). One of the aspects of attitudes is that they involve the cognitive (a knowledge about the object), affective (a feeling about the object), and behavioral (a tendency-toward-action) (Reid, 2006). These three components are closely linked together, but the balance between cognitive, affective, and behavioral can vary (Kind et al., 2007; Reid, 2006).

In science education, the term "attitudes" can be classified into two broad categories: *scientific attitudes* (e.g. open-mindedness, honesty, skepticism) and *attitudes toward science* (e.g. interest in science, attitudes toward scientist, and attitudes toward social responsibility in science). In the first category, this description is inapplicable: traits such as open-mindedness and honesty are better described as styles of thinking which scientists are presumed to display (Gardner, 1975). In the second category, there is always some distinct attitude object (such as science, science lessons, laboratory works, and so on) to which the respondent is invite to react

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favorably or unfavorably. It can be defined as a learned predisposition to evaluate in certain ways objects, people, actions, situations or propositions involved in learning science (Gardner, 1975; George, 2000).

Since students' positive attitudes toward science and other subject matters may be casually linked to ability to learn and achievement (Haladyna, Olsen & Shaughnessy, 1982; Willson & Lawrenz, 1980), factors influence students' attitudes toward science have been revealed. Research studies on the relationships between student attitudes and teacher and classroom characteristics seem to indicate that student attitudes may be associated with classroom learning environment, instructional, teacher-oriented instruction, and teacher believes. Student attitudes toward science seem to be enhanced by harmonious, challenging, and satisfying learning environment (Gabel & Rubba, 1979; Hasan, 1985; Willson & Lawrenz, 1980). Student attitudes toward science seem to improve through the use of some instructional procedure and models. The use of activities-centered, independent study and laboratory work are some of the instructional techniques used to create positive students attitudes toward science (Crawley & Coe, 1990; Hasan, 1985).

2.2.4.2 Students' Perception of Science Learning and Laboratory Work

Although the laboratory experiment can be used to improve students' interest in science (Gardner & Gauld, 1990), students did not understand purpose of that laboratory. They saw laboratory activities as an experience aimed at anticipated results and planed their experiment (Zion, Slezak, Shapira, Link, Bashan, Brumer, Orian, Nussinowitz, Court, Agrest, Mendelovici & Valanides, 2004). Most students viewed their laboratory as the proof of the truth of what they were told in their lecture. They thought that the purpose of laboratory work is to reconceptualize as a process of description through careful data collection. They assumed that they were learning only the scientific method, not a scientific way of thinking since the arbitratory activities which follow a cookbook format emphasize the message that students should be concerned with procedure (Edmoson, 1989). Students framed their data interpretation during practical work in terms of things that can be observed rather than scientific

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theory, they viewed data collected in an experiment as being untainted by the theoretical commitments of the investigators rather than evaluating knowledge claims and data (Leach, 1998; Ryder, Leach & Driver, 1999; Lunetta, 1998). Students thought that problems they encountered with obtaining a good data set were not their fault as long as they followed the relatively explicit procedural instructions. In the classroom laboratory, students often copied an old laboratory report since it can be used in much the same way that one would ask a fellow student for help (Carlo & Bodner, 2004).

Students perceived the science classroom as a place where authority delivers facts (White, 1988) and the laboratory as a place where they were active (in the sense of "doing something") (Hodson, 1998). Although students enjoyed laboratory activities, many students fail to see the connection between what they are doing in the laboratory and what they are learning in the lecture (Havdala & Ashkenazi, 2007; Hodson, 1998). Although their understanding of the methodology required for "doing science" was adequate, most students seemed to have no understanding of the way in which scientists' thinking evolves and is translated into formalized inquiry (Edmondson, 1989). Students did not see science as scientists' efforts to construct explanations for the phenomena in the real world, and did not understand that knowledge is the product of a never-ending process by which it is re-examined and updated (Zion et al., 2004). They were unable to meaningfully summarize the important aspects of an experiment they have just completed (Bell, Blair, Crawford & Lederman, 2003). Students did not recognize that the purpose of some tasks in practical work is to consider the relationship between knowledge claims and data (Leach, 1998). They did not recognize the interplay between data and theory in the process of investigation, and as a result, they may not accept that it was legitimate to develop the design of an experiment in the light of data already collected (Bell et al., 2003; Havdala & Ashkenazi, 2007).

The literature reviews of science education reveal several reasons for these problems. One reason is that most teachers felt constrained to prepare students for tests and examinations and cover science content from textbooks, rather than being concerned with the extent to which students understand what they are to learn (Tobin,

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Kahke & Fraser, 1990). Another is that laboratory work was separated physically from the lecture giving the theoretical principles and concepts it illustrated and the connection may not be evident (Boud et al., 1986; Havdala & Ashkenazi, 2007). The last may be the tradition laboratory class, where students were to a large extent occupied by doing measurements and manipulating apparatus, did not offer much opportunity for students to discuss theoretical knowledge and relate theory to practice (Niedderer, von Aufschnaiter, Tiberghien, Buty, Haller, Hucke, Sander & Fisher, 2002).

Although inquiry-based laboratory has been proposed as an effective tool to develop an understanding of the nature of science by doing science and to relate theory to practice (NRC, 1996), simple engaging in inquiry is not enough to develop student's ideas about these (Lederman, Wade & Bell, 1998). Science educators suggest that an inquiry-based laboratory whose primary focus is to teach specific science concepts should has an epistemological focus (DeBurman, 2002; Leach, 1998; Leach & Scott, 1998; Ryder & Leach, 2006). Students should know epistemology in order to inquire effectively into scientific problems, to understand their inquiry as science, and to be able to evaluate scientific claims in relation to socioscientific issues in their lives (Sandoval, 20005; Sandoval & Rieser, 2004).

2.2.4.3 Epistemological Views for Learning Science

The meaning of epistemology relied on here is nicely captured in two questions frames by Duschl and Osborne (2002) as central to the epistemological basis of science, or really any discipline: How do we know what we know? and Why do we believe it? These questions capture essential facets of the nature of knowledge and its production that are at the heart of inquiry. They include questions about the forms that knowledge may take, including distinctions between laws, theories, and facts, for instance. They also entail questions about both the status and scope of knowledge claims, and the methods for generating knowledge and for evaluating claims (Sandoval, 2005).

Research studies have clearly shown that the epistemological beliefs or the beliefs about nature of knowledge and learning plays a crucial role in determining

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whether and how students coordinate knowledge or theory and empirical evidence in their laboratory practice (Havdala & Ashkenazi, 2007; Smith & Wenk, 2006). Students who preferred using rote learning strategies that were related to positivist views of science and a view of learning as receiving information did not integrate or elaborate cognitive structures (Hogan, 2000). Students who preferred using meaningful learning strategies that were related to constructivist views were actively relating new knowledge to existing frameworks (Hogan, 2000). In addition, students who had performed the inquiry-based laboratory could easily describe the experiment, evaluated or suggested changes and came up with a new experiment objective. They knew what they were doing and had knowledge of the theory connected with the experiment (Berg, Bergendahl & Lundberg, 2003).

Consequently, learning science might be better be characterized as a process of enculturation into a particular "way of knowing", rather than the individual "making sense" of the natural world in their own terms (Leach & Scott, 1998). In other words, to learning about what we know in science, the instructional strategies need to develop learners' capacities to understand how we know and why we belief what we know (Jiménez-Aleixandre, Rodríguez & Duschil, 2000). Students now should study something about the ways in which scientist works and the methods by which knowledge is created and validated (Hodson, 2003; Jiménez-Aleixandre et al., 2000). Teaching and learning methods should be extended in interesting new directions: there should be much less emphasis on the acquisition of factual knowledge via direct instruction and correspondingly greater emphasis on active learning, it should provide more opportunities for students to engage in their own scientific investigations (Hodson, 2003).

According to Sandoval (2005), the constructivist epistemology that should be emphasized on teaching and learning of science can be classified into four broad themes: science as construct, diversity of scientific methods, forms of scientific knowledge, and certainty of scientific knowledge.

Science as construct - The most important epistemological notion for learning science is that scientific knowledge is constructed by people, not simply discovered out in the world (Déautels & Larochelle, 1998; Sandoval, 2005). Indeed, science may

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be best characterized as the effort to explain observations of the natural world, as well as to interpret the experimental manipulations of the world, and therefore, scientific knowledge is based on and/or derived from observations of that natural world (Abd-El-Khalick & Lederman, 2000; Schwartz, Lederman & Crawford, 2004). Human creativity plays an important role in the development of scientific knowledge, and that knowledge is not accepted because it is "true" but because people are persuaded of its value (such as its adequacy as an explanation, or its utility, or some other standard) (Sandoval, 2005). Related to this is the notion that scientific knowledge is socially constructed. Epistemologically, these social aspects of scientific practice are most important in the way they locate criteria for scientific knowledge claims within social, historical communities. That is, scientific claims gain their authority through their ability to persuade (Abd-El-Khalick & Lederman, 2000; Déautels & Larochelle, 1998; McComas, 1998; Schwartz et al., 2004).

Learning from this perspective is viewed as a self-regulatory process of struggling with the conflict between existing personal models of the world and discrepant new insight (Fosnot & Perry, 2005; von Glasersfeld, 1998). That is the individual learn or construct the new knowledge through assimilation, accommodation, and organization. New information is initially assimilated by existing mental structures. If it cannot be understood in terms of the existing mental structure, accommodation take place – that is modified structure evolves - in order to re-establish new equilibration (Fosnot & Perry, 2005). In this context, it is necessary to emphasize that the most frequent source of re-equilibrium for the developing cognitive subject is the interaction with others members of a community (Cobb, 2005; von Glasersfeld, 1998). Related to this perspective, constructivist teachers should promote group learning, a practice that lets two or three students discuss approaches to a given problem (von Glasersfeld, 1998).

Diversity of scientific methods - Part of the diversity in scientific method stems from the differences among scientific disciplines as they explore different kinds of phenomena (McComas, Cloug & Almazroa, 1998). Students should learn that there are many forms of scientific research, that observer bias is a threat to interpretation, and that different researchers using different methods can obtain different results

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(Chin & Malhotra, 2002; Déautels & Larochelle, 1998; McComas, 1998). It has been common to interpret scientific thinking as controlled experimentation to test hypotheses. Controlled experimentation is certainly an important means of generating scientific knowledge (Sandoval, 2005). In addition, scientific fields appear to rely on standards for evaluating methods and the knowledge they produce according to criteria related to systematic care, fit with existing knowledge, and so on (McComas et al., 1998). The main scientific objective is that claims about the natural world have to fit with and make sense of observations of that world (Abd-El-Khalick & Lederman, 2000; Leach, 1998). In the epistemological view for learning science, the goal is to help students develop standards for evaluating the fit between observations, methods of obtaining them, and the knowledge claims advanced through them (Leach, 1998).

From the constructivist perspective, learning and knowledge are instrumental in establishing and maintaining the cognizing subject's equilibrium: they are adaptive. It must be understood as the condition of fit or viability within external and internal constraints. Constraints, however, effect a negative selection. They block and thus determine what does not fit. They do not prescribe the character of what does not collide with them and therefore slip through (von Glasersfeld, 1998). It is noted that a given task has more than one solution and more than one way of arriving at it. The teacher would come to realize that what they present as a problem may be seen differently by the student. Consequently, the student may produce a sensible solution that makes no sense to the teacher. To be then told that it is wrong is unhelpful and inhibiting (even if the right way is explained), because it disregards the effort the student put in. Indeed, such bleak corrections are bound to diminish the student's motivation in further attempts. In contrast, constructivist teachers should adapt instructional activity so that it provides student opportunities for accommodation (von Glasersfeld, 1998). Thus, the constructivist epistemological dimension of learning addresses a process of conceptual restructuring in which students evaluate new knowledge by using evidence that supports or conflicts with their prior ideas (Kang, 2007).

Forms of scientific knowledge - One epistemological view for learning science is that there are different forms of scientific knowledge, varying in their explanatory or

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predictive power and in their relation to the observable world (Sandoval, 2005). According to epistemology for learning science, scientific knowledge may be presented in hypotheses, theories, laws, and models. There are also rhetorical forms, such as explanations, predictions, and arguments (Sandoval, 2005).

Most students appear to believe that hypotheses, theories, and laws are related in a linear hierarchy from less to more proof. That is, hypotheses are guesses, theories are hypotheses supported by evidence, and laws have been irrefutably proven (Sandoval & Morrisson, 2003). Students also seem to think of scientific models in a common every day sense, as physical replicas of some aspect of the world, rather than conceptual systems for explaining phenomena (Grosslight, Unger, Jay & Smith, 1991).

Overall, constructivist epistemology for learning science has objective to lay students to see the relationship between theories, laws, and hypotheses, as well as to understand how a theory differs from a laws or a model. Related to this notion that there is a linear progression from hypotheses to theories to laws, where the difference between these lies in the certainty with which they can be believe (Abd-El-Khalick & Lederman, 2000; McComas et al., 1998). Hypothesis in science may lead to either new theories or to laws with the accumulation of substantial supporting evidence and acceptance in the scientific community. However, theories and laws do not progress one into the other (Déautels & Larochelle, 1998; McComas, 1998) since theories and laws are different in both scope and purposes (Sandoval, 2005). Laws are typically understood as generalized descriptions of some phenomena with high predictive value but little explanatory power. Theories, in contrast, are conceptual frameworks that provide relative high degrees of explanatory power and varying degree of predictive values (Abd-El-Khalick & Lederman, 2000; McComas et al., 1998). In other words, laws describe relationships observed or perceived of phenomena in nature, whereas, theories are inferred explanations for natural phenomena and mechanisms for relationships among natural phenomena (Schwartz et al., 2004). For example, Boyle's law provides a regular description of the relations between pressure, volume, and temperature in containers, while the relations are explained by kinetic molecular theory (Sandoval, 2005).

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Beside theories, laws, and hypothesis, the scientific models seem to produce a useful description correspond to natural phenomena (Leach, 1998; McComas, 1998). However, it should be noted in mind that models are not present reality but they permit prediction and explanation (McComas, 1998).

Certainty of scientific knowledge - It is commonplace in researchers into the nature of science to assert that a sophisticated epistemological viewpoint acknowledges that scientific knowledge is tentative (Abd-El-Khalick & Lederman, 1998, 2000; McComas & Olson, 1998). Scientific knowledge, according to constructivist epistemology, is based on the assumption that reality exits but cannot be known as a set of truth. It is invented, at least in part, to answer questions about the natural world and get as close to the truth as possible, but no bell rings or light blinks to tell scientists that they found the truth (McComas, 1998).

The issue of tentativeness of scientific knowledge stems from the creation of that knowledge through empirical observation and inference (Schwartz et at., 2004). Its product must be valid. A hallmark of science is that it is subject to revision when new information is presented (McComas, 1998). As new data are considered and existing data reconsidered, inference may lead to change in existing scientific knowledge (Schwartz et al., 2004). Scientific knowledge is influenced and driven by the currently accepted scientific theories. The development of questions, investigations, and interpretations of data are filtered through the lens of current theory (Schwartz et al., 2004). This allows science to progress from hypothesis to new knowledge – that is theory or laws (Lederman, Abd-El-Khalick, Bell & Schwartz, 2002; McComas et al., 1998; Schwartz et al., 2004). Hypotheses may lead to either new theories or to laws with the accumulation of substantial supporting evidence and acceptance in the scientific community (Déautels & Larochelle, 1998; McComas, 1998), but will never prove those laws and theory to be true (McComas, 1998).

However, this assertion seems to be dangerous for teaching and learning of science. It seems to be wrong way as if the teacher teach students that scientific knowledge is tentative. Students may not believe or respect with the knowledge since it is not known to be absolute true (Sandoval, 2005). In addition, as Coll and Taylor (2001) note that the teacher may be set one's mind on teaching highly verified

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scientific theories since they can never be totally proven. Although an important instructional goal for teaching and learning of science is the recognition that the current scientific knowledge may change as new observation or new competition ideas come to light and this changing nature of theories reflects the cultural historical development of scientific theories (Sandoval, 2005), it should be noted that this is not easily change. The changing of scientific knowledge may take long time as new observations can prove that the existing knowledge is wrong (Coll & Taylor, 2001). It should also be recognized that scientific knowledge is made from creative acts done by fallible humans. Their conclusions depend on the interpretation of evidence and the more confidence base on their results, and therefore, the conclusions may valid. Of course, it is important to pay attention to how scientific knowledge was generated, but not pay attention only on proving something to be wrong (Coll & Taylor, 2001). Thus, it is important to notion that scientific knowledge varies in its degree of certainty for inquiry in order to remove of absolute certainty decentres authority with respect to knowledge from teacher towards students, and to enable a more authentic consideration of the locus of authority of claims in term of the ability to satisfy epistemological criteria. Some claims are better than others (Sandoval, 2005).

Following points relating to constructivist epistemology views of learning science, it is summarized that scientific knowledge is constructed rather than discovered, and is socially validated. As such, learning science concepts is an adaptive and a cognitive process. It involves introduction of new knowledge through active learning activities and the social process, rather than making sense of the natural world through personal observation and thought. Individuals often have existing ways of knowing about the natural phenomena that are explained by the theories of science. Although the terms used may be the same, the underlying patterns of reasoning are usually different. That is because of existing knowledge and belief. Therefore, teaching and learning of science should promote both declarative (knowing that) and procedural knowledge (knowing how) (Germann, Haskins & Auils, 1996). Declarative knowledge comprises the facts that we know; procedural knowledge comprises the skills we know how to perform (Lawson, 2001). The acquisition of declarative knowledge is very much a constructive process that makes use of procedural

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knowledge, that is, learning new concepts is not a purely abstractive process. Rather, concept learning depends in part on the ability to generate and test ideas and reject those that lead to contradictions (Lawson, 2001; Odom & Kelley, 2001). The learning of declarative knowledge becomes more meaningful and retentions more complete when students participate in the constructive process. Thus, in turn, the procedural knowledge will give students the tools to better understanding and the ability to explain the world by being able to generate and test their own ideas. So, concept construction becomes easier (Lawson, 2001; Odom & Kelly, 2001). Such this approach may have potential to promote students understanding of enzyme kinetics which is regarded as difficult topic for student to learn and for teacher to teach.

2.2.5 Enzyme Kinetics at Higher Education

2.2.5.1 University Student Understandings of Enzyme Kinetics

Enzyme kinetics is an extremely important concept in any biochemistry course (Boyce et al., 2004; Isola & Franzoni, 1998). This is because enzymes are very important chemicals in cells that are used to speed cellular reactions to rates that allow the cell to function properly and survive (Venville, 2008). It is essential to understand enzyme activity in order to understand how life can exist (Kraut et al., 2003). The information about enzyme kinetics is used to interpret the roll of particular enzymes in metabolic control (Leach, 2002). The enzyme kinetics has found applications in the pharmaceutical industry, food, and beverage industries, and industries such as cosmetics, fine chemicals, flavors, and fragrances (Sime, 1999).

A review of science education indicates enzyme kinetics is difficult for students to understand (Logan, 1984; Leone, Baranauskas & Ciancaglini, 1995; Waelbreck, 2006), but there are several empirical data available on students' conceptual understanding of enzyme kinetics. However, some concepts of enzyme kinetic (such as reaction rate and the influence of physical and chemical environment on the reaction rate) are similar to some concepts of chemical kinetics, and therefore, students' difficulty in understanding of enzyme kinetics may be similar to those of

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chemical kinetics which has already observed. Thus, students' difficulties in understandings of chemical kinetics are also reviewed.

Science education research studies reveal that many tertiary students did not understand about the rate of chemical reaction. Students did not know how the reaction rate could be collected (Leach, 2002; Vicario, Casati & Iglesias, 1997). They cannot provide appropriate explanation for the relationship between reaction rate and time. They also have alternative conceptions and difficulties in explaining how reaction rate changes as reaction progress. Students had difficulties in understanding that reaction rate has the highest rate at the beginning of the reaction and the lowest rate at the end, rather they thought the opposite (Cakmakci, Donnelly& Leach, 2003; 2006; Waelbreck, 2006). They seemed to confuse the reaction rate and amount of product. They might have thought that at the end of the reaction, the reaction rate is constant and no product is formed. In addition, students did not generally use a scientifically acceptable concept of reaction rate across context. Even though they can provide appropriate explanation for the phenomena in written or oral form, they fail to construct a symbolic representation for relationship between rate and time or factors that affect the rate (Cakmakci et al. 2003; 2006).

The effects of factors on the rate of chemical/enzyme reactions (such as pH, substance concentrations, temperature, and inhibitor) are one topic that difficult for tertiary students to understand. Many students assume that as long as certain factors are not altered, reaction rate remains constant during the reaction (Cakmakci et al., 2006). They did not know what the optimum pH of enzyme is and the effect which changing pH from the optimum has on enzyme rate reaction (Garatt et al., 2000; Watters & Watters, 2006). They had difficulties in understanding the relationship between concentration of substances and reaction rate (Cakmakci et al., 2003; 2006), they tend to believe that the rate they observed at the highest substrate concentration used is equal to V_m (Waelbroeck, 2007). Students did not know what the range of values of substrate to be used to calculate K_m and V_m (Garratt et al, 2000). Although, most students seemed to know definition for K_m, few referred to how K_m indicates something about binding (Leach, 2002). In addition, many students cannot use

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appropriate reason to explain the effect of temperature on reaction rate (Driel, 2002; Cakmakci & Leach, 2005).

Many tertiary students did not understand the effect of inhibitor on enzyme catalyzed reaction. They thought that the specific inhibited enzyme reaction will cease but other reactions before or after the point of enzyme inhibition will continue (Grayson, Anderson & Gail, 2001). They also do not have clear understanding the types of inhibitors. One reason of this difficulty may be the only available currently mental picture is that of a "competitive inhibitors". Virtually all students immediately picture competitive inhibition (Ocsh, 2000).

The enzyme kinetics models - Michaelis-Menten and Lineweaver-Burk models - are also hard for tertiary students to understand (Leach, 2002; Runge et al., 2006). Although students could relate data about the behavior of enzymes to the Michaelis-Menten model, they thought that Michaelis-Menten model was a perfect description of the kinetics of enzymes, rather than a model of the kinetics of some enzymes under defined conditions. They also thought that the Lineweaver-Burk methods were algorithm, which yields perfect descriptions of the kinetics of an enzyme.

The confusion between chemical kinetics and thermodynamics were observed (Cakmaki & Leach, 2005). Student difficulties are mainly related to mathematical problems arising from differentials in the rate equations and to the inter-relation of enzyme kinetics with thermodynamic (Justi, 2003). One of the reasons would be that these concepts were often taught separately and that the relationships or the differences between those concepts were not specified or ignored in the curriculum. Thus students failed to move between them (Cakmaki & Leach, 2005).

2.2.5.2 Teaching and Learning of Enzyme Kinetics

Although a review of science educational research indicates that enzyme kinetics is a difficult topic area for students to learn and for teacher to teach (González-Cruz et al., 2003; Vicario et al., 1997), few research studies are available on the problems of teaching and learning of this topic. One problem may be the descriptions of enzyme in textbooks used in teaching and learning the enzyme concept are at a molecular level and often students are not up to the abstract thinking necessary

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to understand them (Lazarowitz & Lieb, 2006; Marini, 2005). The other problem is that the abstract manners of teaching and the scant important given to the contribution of mathematical formulas, are legitimate criticisms of the teaching of this subject (Leone et al, 1995; Marini & Ipata, 2007).

Teaching and learning of enzyme kinetics in university is built on student prior knowledge since this topic is taught firstly in secondary school (Justi, 2003; Lim, 2002; Rowe & Brown, 1988), it was expected that secondary school students should have an understanding of the dynamic nature of the reaction system in terms of some fundamental models in enzyme catalyzed reaction (Justi, 2003). During the university level, this idea is reinforce and developed, and more emphasis is given to practical work on enzyme kinetics. The empirical framework of enzyme kinetics (e.g., empirical techniques for measuring reaction rates) and the concepts that make up this empirical framework are introduced in more depth and explained mainly at a mathematical level (e.g., accounting for Michaelis constant). It was expected that tertiary students should be able to develop an understanding of the links between theoretical aspects of enzyme kinetics and experimental data, and to evaluate information and evidence gathered from experiments (Justi, 2003; Lim, 2002; Runge et al., 2006).

Teaching and learning of enzyme kinetics in universities is commonly conducted in both lecture and laboratory. The basic theoretical principles of enzyme imparted by course lectures are often demonstrated and reinforced by undertaking the laboratory activities (Boyce et al., 2004). In such enzyme kinetics laboratories, students perform the experiment by following the protocols in order to attain a predetermined objective (Pich-Otero, Molina-Ortiz, Delaplace, Castellani, Hozbor, Sorgentini & Lodeiro, 1998). Through the correct application of techniques, the experimental data obtained should fit with the predicted results. The experimental observations are then used to revisit the theoretical concepts, from a point of view that now provides new insights based on real world situations (Pich-Otero et al., 1998).

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2.2.5.3 Enzyme Kinetics Experiment at Higher Level

A wide variety of enzyme kinetics experiments have been designed by many science researchers to gives student experiences with enzyme activity measurements, or determination of enzyme kinetics parameters (Hamilton, Dobie-Galuska & Wietstock, 1999; Obón, Castellar, Iborra & Manijón, 2000). For example, Lin and Lloyd (2006) suggested the use of laccases for general chemistry laboratory to give students experience in measuring the enzyme activity as well as zero-, first- and second order reactions. Cornely, Crespo, Errley, Kloter, Levesque and Pickering (1999) suggest the uses of papain to be used for undergraduate biochemistry laboratory to give students experience in measuring kinetics parameter such as K_m and V_{max}.

Haloperoxidases also be an enzyme used for students laboratories. Because of the wide biological distribution of the haloperoxidases and their importance in different system as mentioned above, Jervis, Jervis and Giovannelli (2005) have found the halopeoxidases to be ideal enzymes to engage the interests of the majority of students from a variety of backgrounds. The haloperoxidases, therefore, have been reported to be used to illustrate reaction of environmental and biomedical importance for bioscience students from first-year to final-year.

In summary, these enzyme kinetics laboratory experiments are commonly carried out using spectrophotometer, and use highly purified enzyme and available commercial substrates. The enzyme kinetics experiments at higher level normally focus on measuring of rate reaction and the effect of enzyme and substrate concentrations, temperature, pH, and inhibitors upon enzyme activity (Hardee et al., 2000; Sorensen & Novak, 1996; Vullo, 2003). Most enzyme kinetics experiments have pedagogic value in reinforcing the concepts of Michaelis-Menten kinetics covered in the lecture portion of the course and give students the experience of calculating reaction rate and/or kinetic constants from data (Corneley et al., 1999; Lin & Lloyd, 2006). They also provide practice in important laboratory skills, including: use of a simple spectrophotometer, accurate pipetting, calculation of dilutions and of rate reaction, and data analysis including non-linear curve fitting (Price & Newman, 2000). However, there has been no systematic research into the use and evaluation of these

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enzyme experiments on students' conceptual understanding (Justi, 2003). In addition, most enzyme experiments use special instrument such as spectrophotometers, and require highly purified enzyme and substrate reagents which are expensive. Economical enzyme experiments are needed for student laboratories (Pitch et al., 1998).

2.3 Summary

According to a review of scientific and science educational research, a constructivist epistemology is one of foremost perspective in teaching and learning of science. The learning cycle approach is one of teaching and learning models basing constructivist epistemology. In the learning cycle approach, the laboratory experiences are employed to develop concept. In contrast to the learning cycle approach, traditional laboratories commonly follow a cookbook styles to provide experience with the concept already learned in the lecture. This cookbook format fails to motivate the meaningful intellectual engagement and it is claimed to be an ineffective means of developing students understanding of scientific concept. Although students enjoy in doing laboratory, they fail to see the connection between evidence and theory and they do not understand how scientists develop scientific knowledge.

Inquiry-based laboratories have been proposed to develop student's learning for understanding and to relate theory to practice. Laboratory work whose primary focus is to teach specific science concepts also has an epistemological focus. Students now should study not only conceptual knowledge (knowing that) but also procedural knowledge (knowing how). The learning cycle is one of inquiry-based laboratory that provides students with experiences in generating both conceptual and procedural knowledge. This approach has been proved very effective to promote conceptual understanding, to develop process skills, to improve thinking and reasoning abilities, and to create positive students attitudes toward science and laboratory.

Therefore, the learning cycle approach may give students in meaningful learning and understanding the difficult concept as enzyme kinetics. Since many research studies indicate the learning cycle is an effective approach in enhancing conceptual understanding, improving science process skills, improving thinking and

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reasoning abilities, as well as creating positives attitudes toward science and inquiry, these approach may have potential in promoting Thai tertiary students to understand the enzyme kinetics. However, not only the teaching strategies, but also economical enzyme kinetics experiment needs to be developed to promote student understanding of enzyme kinetics. Therefore, the enzyme kinetics experiment that uses local materials is needed.

Regarding the seaweed that contains bromoperoxidases which have great promise for biotechnological applications, the seaweed is attractive to be used as source of the enzyme for student's laboratory. In the assay bromoperoxidases activity, various substrates have been used. However, the traditional substrates such as monochlorodimedone and phenol red are not being a good substrate for assay the bromoperoxidase activity in student's laboratory. The assay using monochlorodimedone requires an expensive instrument as UV-spectrophotometer for detection. The assay using phenol red causes lag time. Bromophenol red has been suggested to be used for assay bromoperoxidase activity. The reaction using bromoperoxidase as substrate may eliminate lag time problem and it can be detected by using a simple spectrophotometer. Therefore, bromoperoxidase may be a better substrate for assay the bromoperoxidase activity.

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CHAPTER III
METHODOLOGY AND METHODS

Overview

This chapter describes methodology and methods used to conduct the research to answer the research questions posed earlier. It begins with the theoretical basis and the theoretical framework of the research. Then the research methodology design and methods used for conducting this research are described. Next, the participants involving this research are specified. Finally, the methods/tools used for collecting and analyzing data are described.

3.1 Theoretical Basis and Theoretical Framework

According to higher education reform in Thailand, it requires teacher to adopt active learning strategies and the teaching should be learner-centered (Shawyun, 2005; ONEC, 2003; OCS, 2002; MOE, 2004a). The constructivist-based teaching and learning is introduced to Thai curriculum in order to develop students' lifelong learning with a balance of knowledge, skills, and attitudes (Dahsah & Faikhamta, 2007; MOE, 2003). Related to this notion, the constructivist learning theory is used in this study.

Not only constructivist learning theory, this study has also been derived from the literature which suggests that teaching and learning of science whose primary focus is to teach specific science concepts should has an epistemological focus (DeBurman, 2002; Hodson, 2003, Leach & Scott, 1998; Ryder & Leach, 2008). This has been proved very effective to promote student to inquire effectively into scientific problems, to understand their inquiry as science, and to be able to evaluate scientific claims in relation to socio-scientific issues in their lives (Hodson, 1992; Sandovol, 2005). Therefore, the constructivist epistemology was also served as one of the framework of this study (see the left part of Figure 3-1).

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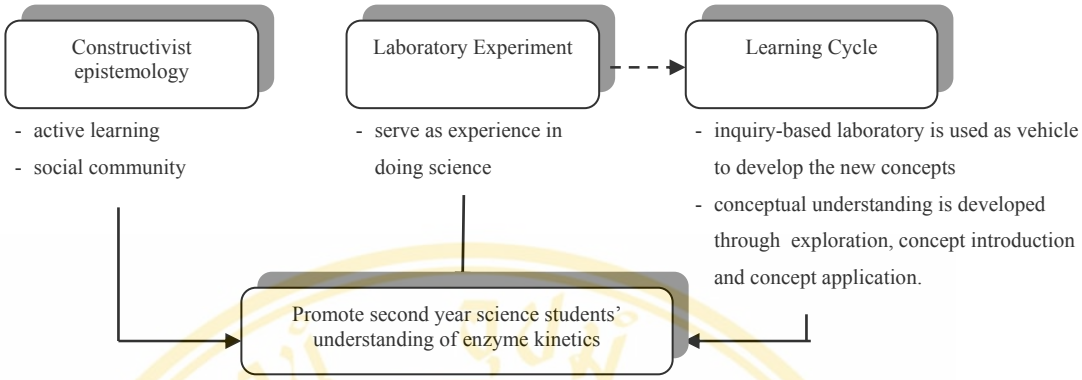

Figure 3-1 Theoretical Framework

As mentioned in the literature review, the central to constructivist epistemology is a view about the nature of human knowledge and more particularly of scientific knowledge; a view about the origins, transmission mechanisms, and validation procedures of scientific knowledge (Matthews, 1994). The researcher considered that students were purposeful in their learning, and that they actively constructed knowledge in their own minds, rather than acted as passive recipients of knowledge. The development of scientific knowledge was not only constrained by empirical data, but was also socially validated by the scientific community and goes beyond descriptive accounts of the natural world. Scientific knowledge cannot, therefore, be learnt from sensory experience alone (Leach & Scott, 2003). This study's design assumed that the best teaching in science was learning driven, and occurred when students had more time to explore and experiment with phenomena and hence encouraged learners to develop new knowledge schemes that were better adapted to experience (see the middle part of Figure 3-1). The experiments or practical work, according to the leaning cycle approach, was considered to be used as experience for new concepts and insights to interact with the students' own initial conceptions, and then to apply the concepts to a new situation (see the right part of Figure 3-1). In a class taught by inquiry, individuals were actively engaged with others in attempting to understand and interpret phenomena for themselves, and social interaction in groups provided the stimulus of differing perspectives for reflection. It was decided that an inquiry-based laboratory that places students in small research teams for designing experiments to investigate a particular question posed by the instructor is the best

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strategy to introduce students to the way of knowing science. Students must make important decisions concerning the experimental set-up, collection of relevant data, its interpretation, and judgements regarding the veracity of their work. Ensuring negotiation of meaning conveys a very different picture of science to the typical cookbook/verification laboratory work where students are following recipes to arrive at preordained results. The teacher's role was to provide the physical experiences and acted as facilitator for student construction of knowledge, and helped students learn meaningfully, to achieve quality over quantity and meaning over memorization.

The researcher believed that the learning cycle approach, such as that described in this work, was best addressed to engage students in meaningful inquiries and had the aim to help students construct meaningful concepts since this approach provided students with experiences in generating both declarative and procedural knowledge (Lawson, 2001, 2003). It has been found in science education research to be an effective method that promoted students understanding of scientific concepts, reduced misconceptions (Lawson, 2001), improved student's thinking and reasoning abilities (Musheno & Lawson, 1999; Lawson, 1986; 2001), as well as creating positive attitudes toward sciences and scientific inquiry (Bevevino et al., 1999).

Although there were many phases in the learning cycle approaches (4E, 5E, and 7E), the researcher has chosen to use the phases of exploration, term or concept introduction, and concept application described by Lawson (1986, 2001) because they were the foundation phases developed most closely related to the pioneer in the learning cycle (Robert Karplus, early 1960's), and have been proved effective in teaching and learning of science.

As shown in Figure 3-1, the researcher planed to promote student understanding of enzyme kinetics by applying the theoretical concepts of constructivist epistemology, practical work, and the Lawson's learning cycle to develop the laboratory activities to be used in a biochemistry subject undertaken by second year Thai university students. The learning cycle was, as Lawson (1986, 2001) defined, an inquiry-based teaching approach comprising three distinct phases of instruction: (1) exploration, (2) term or concept introduction, and (3) concept application. In Phase 1 (exploration), students would have an opportunity to make

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observations, perform experiments, collect, examine, and analyze data or information, and investigate relationships dealing with the topics being introduced in the classrooms. In the next phase (term or concept introduction), students organized and analyzed data obtained from the first phase, and the teacher introduced new terminology. In the final phase (concept application), students would be encouraged to apply those concepts to solve new problems introduced in the classroom. In this study, the enzyme experiments were used to provide students with experience of measuring enzyme activities and identifying the factors that affect enzyme functions. Students were given opportunities to design and perform experiments by themselves with minimal guidance to investigate the factors that influence enzyme activities. The data from these experiments were shared and discussed to generalize the concepts. Following this, students were given assignments in order to apply that knowledge to new situations. Students were asked to write laboratory reports validating their data and using knowledge to support their results.

To give students experience about this, bromoperoxidase from Thai seaweeds has been used for student laboratory experiments. Regarding the implementation of the instructional design, the research study not only seek to determine the effectiveness of the bromoperoxidase laboratory activities on student understanding of enzyme kinetics, but also investigated how the learning cycle approach based on these experiments influences students' learning.

3.2 Research Methodology

The research design was divided into three stages as follows:

(1) Development of a simple detection for assay of red seaweed bromoperoxidase activity using a new substrate, bromophenol red.

(2) Implementation of an instructional model, the learning cycle based on kinetics assay of seaweed bromoperoxidase, in undergraduate laboratory.

(3) Investigation of the effect of the learning cycle activities based on bromoperoxidase experiment on the student achievements and their perceptions.

These are summarized in Figure 3-2.

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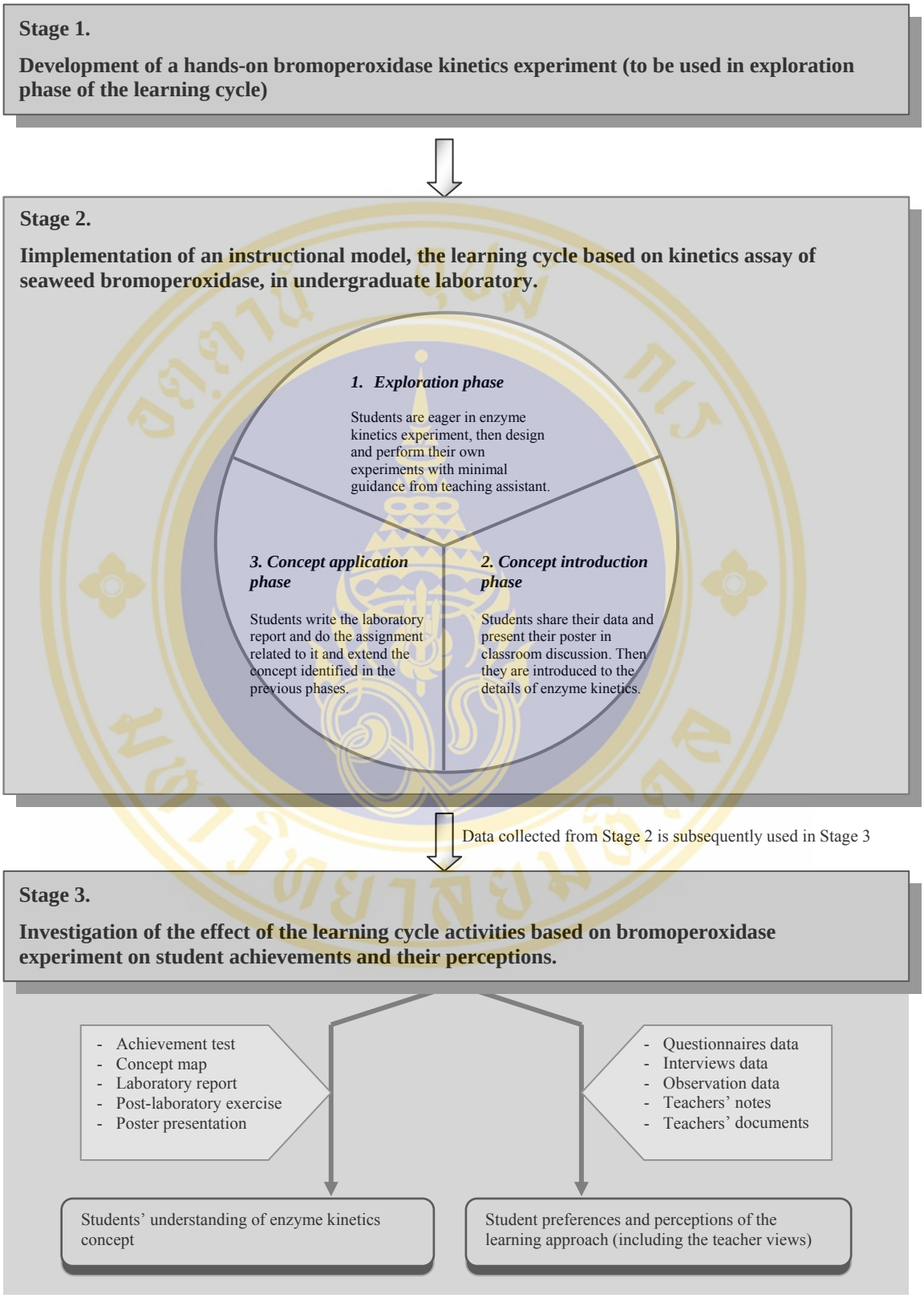

Figure 3-2 Research methodological design

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3.3 Methods

3.3.1 Development of a Hands-on Bromoperoxidase Kinetics Experiment

3.3.1.1 Preparation of Red Seaweed

Thai red seaweeds *(Gracilaria tangi)* (Figure 3-3) were collected from the seashores of Pattani Province and from Kho Yor of Songkla Province. The seaweeds were washed 2-3 times with tap water to remove sand and debris and then washed 2 times with distilled water. The washed seaweeds were frozen at -20^{o}C until use.

Figure 3-3 Red seaweeds *(Gracilaria tangi)*

3.3.1.2 Preparation of Crude Enzyme from Seaweed

The red seaweeds were thawed by soaking overnight in 20 mM Tris-HCl buffer pH 7.0 at 4^{o}C and then homogenized by using the Warring mixing blender with 1:1 w/v (g/ml) of iced-cool 20 mM Tris-HCl buffer pH 7.0. The homogenate was filtered through a cheesecloth and the filtrate was centrifuged at 6,000 rpm for 45 min at 4^{o}C. The supernatant (crude enzyme solution) was collected and kept at 4^{o}C for further assay of bromoperoxidase.

3.3.1.3 Determination of Protein Concentration

Protein concentration was determined by the method of Bradford (Bradford, 1976). Coomassie blue reagent was added into 0.1 ml of protein sample. The absorbance at 595 nm was measured after standing the solution mixture for 10 min at 25^{o}C. Blank contained distilled water instead of protein sample. Bovine serum albumin was used as a standard protein.

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Coomassie blue reagent was prepared by dissolving 100 mg of Coomassie brilliant blue G-250 in 94 ml of 95% Ethanol. Then 200 ml of 85% phosphoric acid were added into the mixture which was then made to a final volume of 2L with distilled water. The Coomassie blue reagent was kept at 4°C for protein determination.

3.3.1.4 *Determination of Bromoperoxidase Activity in Crude Extract*

The bromoperoxidase activity was assayed at room temperature by measuring the increasing in absorption of brominated products (bromophenol blue, Br_4PR) at 590 nm ($\Sigma_{\lambda 590}$ = 70.4 $mM^{-1}cm^{-1}$) (Wong, Hwang & Mou, 2006) for 3 min in the 3 ml reaction mixture containing 12.5 µM bromophenol red, 20 mM KBr, and 0.2 mM H_2O_2 in 0.1 M phosphate buffer pH 6.5 at 25°C. The reaction mixture containing all substances except enzyme was used as control. The enzyme activity (U) was defined by unit per ml of enzyme (µmole Br_4PR/min/ml) (Farrell & Taylor, 2006; Palmer, 1995), calculated as:

$$\text{Enzyme activity (U/ml)} = \frac{A_{590}/\text{min} \times \text{total volume (ml)}}{E^{mM}_{590} \times \text{enzyme volume (ml)}}$$

The specific activity was defined by the activity per milligram of proteins (U/mg) (Farrell & Taylor, 2006), calculated as:

$$\text{Specific activity (U/mg)} = \frac{\text{Enzyme activity (U/ml)}}{\text{Protein concentration (mg/ml)}}$$

3.3.1.5 *Investigation of Br_2PR as Organic Substrate for Bromoperoxidase in Comparison to PR*

Dibromophenol red (Br_2PR) was tested as substrate for bromoperoxidase in comparison with phenol red (PR). For investigation of PR as substrate, 0.5 ml of crude enzyme (0.19 mg protein/ml) was added to the mixture containing 20 mM KBr, 4.17 µM PR, and 0.2 mM H_2O_2 in 0.1 M phosphate buffer pH 5.5 in total volume of 3 ml. The absorption spectra of the reaction mixture were then recorded using UV-visible recording spectrophotometer from 300 nm to 800 nm at 25°C.

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For investigating Br_2PR as substrate, the experimental procedure was performed as described above, but Br_2PR was used instead of PR.

3.3.1.6 Assay of Bromoperoxidase Activity by Measuring the Increasing of Brominated Products

0.5 ml of crude enzyme (0.19 mg protein/ml) was added to the mixture containing 20 mM KBr, 33.33 μM Br_2PR, and 1 mM H_2O_2 in 0.1 M sodium acetate buffer pH 6.5 in total volume of 3 ml. The absorption of the reaction mixture was measured using a simple spectrophotometer (OPTIMA) at 590 nm every 5 sec at 25°C until the absorption does not change (approximately 60 min). The reaction mixture containing all substances except enzyme was used as control. The initial rate of the reaction was obtained from slope of a plot of absorption against time.

3.3.1.7 Optimization of Colorimetric Assay for Red Seaweed Bromoperoxidase

3.3.1.7.1 Determination of pH optimum for bromoperoxidase

Crude enzyme was used for assay bromoperoxidase activity as described in section 3.3.1.5 at various pHs (0.1 M acetate buffer pH 5.5, 6.0, 6.5 or 7.0). The pH optimum was obtained from a plot between initial rates and pH value.

3.3.1.7.2 Effects of enzyme concentrations on bromoperoxidase activiy

Crude enzyme was used for assay bromoperoxidase activity. The assay mixture (total volume of 3 ml) containing 20 mM KBr, 33.33 μM Br_2PR, and 0.2 mM H_2O_2 in 0.1 M acetate buffer pH 6.5 was started by addition of various amount of crude enzyme (ranging from 50 μL to 1,000 μL). After starting the reaction, the absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer. The reaction mixture containing all substances except

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enzyme was used as control. The initial rates at all enzyme concentrations were obtained from slope of a plot of absorption against time.

3.3.1.7.3 Effects of Br_2PR concentrations on bromoperoxidase activity

To investigate the effects of bromophenol red (Br_2PR) concentrations on bromoperoxidase activity, the assay was performed in the mixture (total volume of 3 ml) containing 20 mM KBr, 0.2 mM H_2O_2 in 0.1 M acetate buffer pH 6.5 using various concentrations of Br_2PR (ranging from 4.16 μM to 33.33 μM), and the reaction was started by addition of 0.5 ml of crude enzyme (0.19 mg protein/ml, 0.1 U/mg protein). After starting the reaction, the absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer. The reaction mixture containing all substances except enzyme was used as control. The initial rates for all Br_2PR concentrations were obtained from slope of a plot of absorption against time.

3.3.1.7.4 Effects of KBr concentrations on bromoperoxidase activity

To investigate the effects of KBr concentrations on bromoperoxidase activity, the assay was performed in the mixture (total volume of 3 ml) containing 33.33 μM Br_2PR, 0.2 mM H_2O_2 in 0.1 M acetate buffer pH 6.5 using various amount of KBr ranging from 0.03 mM to 1.67 mM and then the reaction was started by addition of the 0.6 ml of crude enzyme (0.19 mg protein/ml, 0.1 U/mg protein). After starting the reaction, the absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer. The reaction mixture containing all substances except enzyme was used as control. The initial rates for all KBr concentrations were obtained from slope of a plot of absorption against time.

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3.3.1.7.5 Effects of H_2O_2 concentrations on bromoperoxidase activity

To investigate the effects of hydrogen peroxide (H_2O_2) concentrations on bromoperoxidase activity, the assay was performed in the mixture (total volume of 3 ml) containing 20 mM KBr, 33.33 μM Br_2PR in 0.1 M acetate buffer pH 6.5 using various concentrations of H_2O_2 (ranging from 0.1 mM to 2.8 mM), and the reaction was started by addition of 0.2 ml of crude enzyme (0.19 mg protein/ml, 0.1 U/mg protein). After starting the reaction, the absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer. The reaction mixture containing all substances except enzyme was used as control. The initial rates for all H_2O_2 concentrations were obtained from slope of a plot of absorption against time.

3.3.1.7.6 Studies on storage stability of bromoperoxidase

Aliquots of crude enzyme kept at 4°C were taken for assay enzyme activity of different time periods (1-17 days). The enzyme assay was performed by using 20 mM KBr, 33.33 μM Br_2PR, 1 mM H_2O_2 in 0.1 M sodium acetate buffer pH 6.5, and the reaction was started by addition of 0.3 ml of crude enzyme (0.19 mg protein) in total volume of 3 ml. The absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer (OPTIMA). The reaction mixture containing all substances except enzyme was used as control. The activity of enzyme kept for different periods was reported as percentage of relative activity with the freshly prepared enzyme.

3.3.1.7.7 Studies on temperature stability of bromoperoxidase

Crude enzyme (0.19 mg protein) was incubated at various temperatures (ranging from 4°C to 94°C) for 5 min in a water bath. At the end of the incubation, the enzyme was cooled to room temperature for about 5 min and then assay for remaining bromoperoxidase activity by addition of 0.3 ml of the treated enzyme into the mixture containing 20 KBr, 33.33 μM Br_2PR, 1 mM H_2O_2 and 0.1 M sodium acetate buffer pH

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6.5 in total volume of 3 ml. The absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer (OPTIMA). The reaction mixture containing all substances except enzyme was used as control.

3.3.1.7.8 Effects of V_2O_5 on bromoperoxidase activity

Freshly prepared crude enzyme and the enzyme kept for one day were used for investigation the effects of vanadium (V) oxide (V_2O_5) on their activities. The assay was performed by adding various concentrations of V_2O_5 (ranging from 41.67 μM to 333 μM) to the mixture containing 20 mM KBr, 33.33 μM Br_2PR, and 1 mM H_2O_2 in 0.1 M sodium acetate buffer pH 6.5, and the reaction was stared by addition of 0.3 ml of the enzyme in total volume of 3 ml. The absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer. The reaction mixture containing all substances except enzyme was used as control. The enzyme activity or initial rate at various concentrations of V_2O_5 was displayed as percentage of relative activity with freshly prepared enzyme in the absence of V_2O_5.

3.3.1.8 Effects of NaN_3 on Inhibition of Bromoperoxidase Activity

3.3.1.8.1 Effects of NaN_3 concentrations on bromoperoxidase activity

To investigate the effect of sodium azide (NaN_3) on the inhibition of bromoperoxidase activity, NaN_3 at various concentrations (ranging form 0.032 mM to 1.281 mM) was added into the mixture containing 20 mM KBr, 33.33 μM Br_2PR, and 1 mM H_2O_2 in 0.1 M sodium acetate buffer pH 6.5, and the reaction was stared by addition of 0.3 ml of crude enzyme in total volume of 3 ml. The absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer. The reaction mixture containing all substances except enzyme was used as control. The enzyme activity or initial rate at various concentrations of NaN_3 was calculated as percentage of relative activity with the enzyme in the absence of NaN_3.

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3.3.1.8.2 Investigation of mechanism of inhibition by NaN_3 at fixed concentration of KBr

For competition of NaN_3 with KBr, the assay was performed at fixed concentration of KBr (3.33, 6.67, 10.00, 13.33, 16.67 or 20.22 mM) at various concentrations of NaN_3 (ranging from 0.13 to 0.38) in the mixture containing 33.33 μM Br_2PR, and 1 mM H_2O_2 in 0.1 M sodium acetated buffer pH 6.5, and the reaction was started by addition of 0.3 ml of crude enzyme in total volume of 3 ml. After starting the reaction, the absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer. The initial rate of all reactions was calculated. A plot of 1/v (at different concentrations of NaN_3) against 1/[KBr] was performed.

3.3.1.8.3 Investigation of mechanism of inhibition by NaN_3 at fixed concentration of Br_2PR

For competition of NaN_3 with bromophenol red (Br_2PR), the assay was performed at fixed concentration of Br_2PR (8.33, 16.67, 25.00, 33.33 μM) at various concentrations of NaN_3 (ranging from 0 to 0.0.26 mM) in the mixture containing 20 mM KBr, 1 mM H_2O_2 in 0.1 M sodium acetated buffer pH 6.5, and the reaction was started by addition of 0.3 ml of crude enzyme in total volume of 3 ml. After starting the reaction, the absorption of the reaction mixture was measured at 590 nm every 5 sec for 3 min at 25°C using a simple spectrophotometer. The initial rate of all reactions was calculated. A plot of 1/v (at different concentrations of NaN_3) against 1/[Br_2PR] was then performed.

3.3.1.9 Preparation of Agar Gel Slide for Testing Bromoperoxidase

The agar gel slide for testing bromoperoxidase activity were prepared by boiling 50 ml of solution containing 0.5 g agar (available in local supermarket), 0.2 M sodium acetate buffer pH 6.5, 50 μM bromophenol red, and 50 mM potassium bromide. After melting, the agar solution was cooled at room temperature until the solution's temperature was 40°C, then 20 ml of 6 mM hydrogen peroxide were added

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into the solution. About 5 ml of the mixture solution was poured on a microscopic slide and allowed to set at room temperature. The agar gel slides were kept in a cool chamber until use.

3.3.1.10 Bromoperoxidase Activity Testing by Agar Gel Slide

To test bromoperoxidase activity in seaweed using agar gel slide, cut seaweeds or fronds were placed into the agar gel. Change from pink color of Br_2PR around the seaweed to blue color of Br_4PR indicated bromoperoxidase activity. The color appeared at 5-60 minutes depending on the enzyme activity.

3.3.1.11 Development of a Hands-on Kinetics Experiment Using Red Seaweed Bromoperoxidase

A hands-on experiment using red seaweed bromoperoxidase was developed to be used in a basic biochemistry course for second year undergraduate science students. The laboratory experiment was designed to 3 hour. To engage the students' interest in the enzyme experiment, they were given the agar slide containing Br_2PR substrates and the marine red alga *(Gracilaria tangi)* for testing the bromoperoxidase activity. The students were supposed to insert the cut seaweeds into the agar gel and observed the color change.

For kenetics assay of seaweed bromoperoxidase, a group of students was provided with a time counter and graduated pipettes and a simple spectrophotometer, the spectronic 20. Also provided were crude extract of enzyme, 1 M acetate buffers (pH 5.5, 6.5 and 7.5), a solution of Br_2PR at 76.9 mM, KBr at 46.1 mM, H_2O_2 at 2.3 mM and NaN_3 at 3.85 mM. Equipped with the relevant scientific background information on their observed color change in the engagement step, each group of students was allowed to make their own choice to select one of the effectors for the assay, i.e., pH, enzyme concentrations, substrate concentrations or sodium azide concentration. Each groups of students then designed their own experimental procedure on the selected effector.

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3.3.2 Implementation of an Instructional Model, the Learning Cycle based on Kinetics Assay of Seaweed Bromoperoxidase, in Undergraduate Laboratory

The enzyme kinetics laboratory was based on an inquiry-based learning cycle activity. The enzyme kinetics experiment using red seaweed bromoperoxidase was used as a means to enhance concept development rather than to demonstrate and reinforce the concepts students have learned in lecture. It was used for second year undergraduate science students enrolled in the basic biochemistry course. This 3-hour laboratory class and 3-hour lecture class schedule is divided into three phase: exploration, concepts introduction, and concepts application.

During the *exploration phase*, students performed the bromoperoxidase experiment by themselves with minimal guidance from the teacher. The teacher challenged the students with central questions to be investigated and discussed in small groups (e.g. What is enzyme rate reaction? and How is the enzyme reaction rate measured?). Students then engaged in an inquiry activity to observe and collect data to answer the questions. These activities allowed teacher to understand students' prior knowledge. Then each group of students was given the opportunity to design an experiment to investigate factors affecting the enzyme activities: pH, enzyme/substrates concentrations, or some other substances. The data from the experiments could raise questions or complexities that students could not resolve or answer with their present concepts. In other words, these gave opportunities for students to voice conflicting or, at least, partially inadequate ideas which could spark debate and analysis of the reasons for their ideas.

The *concept introduction phase*, gave students' opportunities to participate in the knowledge construction process. This involved students in negotiation and discussion. Groups of student performing the same experiment (or same factor) shared their results with each other. Next, they were given opportunities to present their findings and discuss with the whole class. The social interaction and negotiation could lead to the building and rebuilding of individual's cognitive structures. During this phase, formal terms such as enzyme activity, initial rate (v), maximum velocity (V_{max}), optimum pH, inhibitor and activator were defined. Following the laboratory activity,

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the concepts of enzyme kinetics were taught in details in the lecture. The direct teaching of the concept was attempted after students explore the experiment because this gives a chance for students to explore the experiments and develop some concepts familiar with them since, according to Piaget's cognitive learning theory, the students were expected to experience some cognitive conflict or disequilibrium, and the desire to achieve equilibrium in this phase.

In the last phase, *concept application*, students were asked to write the laboratory report by using knowledge they learned from lecture to support their conclusion, and to answer the related questions and extend the concept identified in the previous phases. These activities provided students' opportunities to use the knowledge they had learned from previous phase to solve the problems or to generalize it to other situations. These gave students, according to Ausubel's idea, meaningful learning.

In this laboratory class, there were five instructors: one biochemistry teacher and four teaching assistants. They acted as facilitators to the groups of students (one teaching assistant per four groups of students). All teachers were instructed to their roles in facilitating student's learning prior to the laboratory activity.

The overall activities is summarized in Table 3-1

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Table 3-1 Instructional steps for enzyme kinetics topic designed for second year undergraduate science students enrolled in the basic biochemistry course

Phase	Student activity	Objective
Exploration (2 hours laboratory class)	Investigate the enzyme activity in seaweeds using agar gel staining for bromoperoxidase activity	To identify enzyme, substrate, and products of the reaction To explain what is going on in the particular reaction To predict the enzyme activity
	Measure bromoperoxidase activity using a simple spectrophotometer	To study the colorimetric method for measuring the enzyme activity To measure the initial rate of enzyme catalyzed reaction To compare the rate of the reaction with or without enzyme
	Design and perform the experiment with minimal guidance to investigate the factor(s) affecting rate of enzyme catalyzed reaction	To investigate the effects of enzyme concentrations, substrate concentrations, pH and sodium azide on the rate of enzyme catalyzed reaction
Concept introduction (1 hour laboratory class)	Compare results with those of other groups and draw a conclusion about the experiment	To interpret results and draw conclusions based on the empirical data
	Prepare a group poster to present the findings and discuss in front of the whole class	To explain the influence of enzyme concentrations, substrate concentrations, pH and sodium azide on the rate of enzyme catalyzed reaction To communicate the scientific knowledge
Concept introduction (3 hours lecture class)	Learn in-depth concepts of enzyme kinetics	To link the new knowledge with the experience in the exploitation phase
Concept application (homework)	Solve problems related to enzyme kinetics experiment	To apply or use knowledge they have learned in new situations

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3.3.3 Effects of the Learning Cycle Activities Based on Bromoperoxidase Experiment on Student Achievements and Their Perceptions

Not only student's conceptual understanding is an important objective of the instructional design, but also student's perception is equal importance because it determines student's achievement (Crawley & Coe, 1990; Neathery, 1997; Wolf & Fraser, 2008). Therefore, this study investigated both students' achievements in understanding of enzyme kinetics, and their perceptions toward the learning approach.

According to Millar et al. (2002), and Psillos and Niedderer (2002), the research design in this study determined the students' achievement during the practical work in two categories: (1) observe the student activities during the given practical work, and (2) measure the learning outcomes after completing the practical work.

Determining the achievement in the first category was based on observation of what students actually do in regard to the use of knowledge during practical work. This involved an analysis of videotape from practical work according to predetermined categories. It focused on student activities in making links between theory and practice that included talking about scientific concepts and/or talking about relations between scientific concepts and real objects. This data was triangulated by results from an audio-recorded of conversation of students or students and teachers, as well as notes of events taken during the practical work.

Determining the achievement of practical work in the second category involved measuring the learning outcomes by using pre/post analysis of tests, pre/post concept maps, analysis of the laboratory reports, and interviews. In addition, data from post-laboratory exercises and poster presentation were also used as supported evidences.

As mentioned above, student perceptions of the learning approach influence their achievement. Therefore, student perceptions, and feedback from both students and teachers about the learning cycle approach were considered. Questionnaires, students interviews, teacher documents (such as teaching plan, laboratory manual, power points, journal notes), and classroom observations were used to investigate how this instructional design worked. In addition, the difficulties or dissatisfactions of students and teachers during the practical work were also considered.

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3.4 Participants

This study was conducted at the public university in the central part of Thailand. Seventy-two second year undergraduate students enrolled in second-semester general biochemistry in 2007 participated in the study. This course involved a 3-hour laboratory session and 3-hour lecture.

In this study, the learning cycle based on bromoperoxidase experiments was implemented to teach the concepts of enzyme kinetics. Students performed the enzyme laboratory before learning the concept of enzyme kinetics from the lecture.

3.5 Ethics

The ethical concerns were principally those of the ongoing maintenance of confidentiality with respect to the data, obtaining informed consent from all participants, monitoring for potential harm throughout the research, and the methods for dealing with any concerns of the participants with respect to being involved in a research. These concerns were addressed throughout the research as well as at the beginning.

It was the responsibility of the researcher to be open about the purpose and methods of the research with all participants. The teachers and students involved in this research were given brief information before the research was undertaken. The aims and questions which formed the basis of the research, and the requirements of the research in terms of data collection were explained. The ethics governing the conduct of the project were outlined. These included procedures the researcher would follow in setting up interviews and observations; ownership of the data (by the teachers and students) and data analysis (by the researcher); in maintaining the confidentiality and accuracy of transcripts; in monitoring for potential harm; the methods for dealing with concerns; and the method for withdrawing from the research. The participants were also asked to sign their names as they agreed to participate in this research. To conduct the research in classroom, the researcher also contacted and informed the university department principal for permission.

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3.6 Data Collection in Educational Research

In educational research study, data collection possibilities are wide and varied, with any one method of collection not being inherently better than any other. Each has its strength and weakness that must be weighed up in view of a rich and complex context. Therefore, the backgrounds, strengths and weaknesses of the methods used for collecting data are reviewed to provide the useful information for conducting this research study.

3.6.1 Questionnaires

Questionnaires are very popular with quantitative researchers (Borg, 1963; Bryman, 1984). They are a useful tool for collecting data from a large number of respondents (Hinds, 2000), and can be used to collect a wide range of data in a variety of different circumstances (Moore, 2000) by delivery in a variety of different ways (Drew, 1980; O'Leary, 2005). The questionnaires provide preliminary and macroscopic understanding of an existing situation (O'Leary, 2005), often numerical data (Cohen et al., 2007).

In constructing a questionnaire, each item must be developed to measure a specific aspect of one of the objectives (Borg, 1963). Responses to survey questions can be either open or closed (Hinds, 2000; Moore, 2000; O'Leary, 2005). In an *open-ended question* or *open response questionnaire*, the respondents are asked to provide answers using their own words. They can offer any information, or express any opinion they wish (Moore, 2000; O'Leary, 2005). The data provided can be rich, but can also be difficult to code and analyze (Lewin, 2005; O'Leary, 2005) since so many different kinds of responses are received (Fraenkel & Wallen, 1993). Besides that, the open-ended response questionnaire has obvious limitations. It is only effective when used by people who are happy expressing themselves in writing (Moore, 2000).

In a *closed question* or *closed response questionnaire*, the respondents are asked to choose from a range of predetermined responses (O'Leary, 2005), which are in various forms such as dichotomous answers, multiple choices, ranking ordering, and rating scales (Cohen et al., 2007). The respondents, therefore, can complete the survey at a convenient time (Cohen et al., 2007). The data here is generally easy to code and

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analyze statistically (Moore, 2000; Lewin, 2005). However, the use of this questionnaire also has limitations. It may lack coverage or authenticity (Cohen et al., 2007; McMillan & Schumacher, 1993).

Language used in a questionnaire is also important. It should be clear, unambiguous, inoffensive and unbiased (O'Leary, 2005). It is highly recommended that researchers should conduct a pretest of their questionnaires before using them in studies (Cohen et al., 2007; Moore, 2000), to ensure that the respondents do not misunderstand the questions or statements posed in questionnaire (Drew, 1980; Hind, 2000), and to assess the time effort required on the part of respondents (Drew, 1980). It is very important that the pretest be conducted with the sample of subjects with characteristics similar to those that will be used in the study (McMillan & Schumacher, 1993), otherwise the pretest will tell little or nothing of how the actual respondents will react (Drew, 1980).

Using questionnaires in the research has both strengths and weaknesses. They are good for collecting data on non-contentious and relatively straightforward topics, but are not good for asking about sensitive issues, or for exploring complex issues or concepts that are hard to defined (Moore, 2000). A questionnaire survey is relatively economical (Borge, 1963) and easy to administer (Moore, 2000, O'Leary, 2005) with a large sample. It tends to be highly reliable, and encourage honesty (Cohen et al., 2007) because it can ensure anonymity (Borg, 1963) since the researcher does not have direct contact with the respondents (Cohen et al., 2007). The questionnaire can be completed at a convenient time (Moore, 2000). It also ensures that all respondents are presented with the same questions without introducing any bias (Moore, 2000). However, there is often a low percentage of return (Moore, 2000). They are also unlikely to produce a great depth of information (Cohen et al., 2007).

3.6.2 Interview

Interview involves the collection of data through direct verbal interaction between individuals (Borg, 1963). The use of interviews may serve three purposes. First, it may be used as the principal means of gathering information having direct bearing on the research objectives (Cohen et al., 2007). Second, it may be used to test

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hypotheses or to suggest new ones, or as an explanatory device to help identify variables and relationships (Cohen et al., 2007). And third, the interview may be used in conjunction with other methods in a research undertaking (Hoepfl, 1997) in order to, for example, validate other methods, or go deeper into the motivations of respondents and their reasons for responding as they do (Cohen et al., 2007).

To conduct interviews, an interview guide or schedule – that is a list of questions or general topics that the interviewer wants to explore during each interview - is required to ensure that basically the same information is obtained from each person (Hoepfl, 1997). During a *formal or highly structured interview*, these set questions are asked in sequence and the answers are recorded on a standard schedule (Hoepfl, 1997). Therefore, it is easy to record but may yield more superficial information (Best & Kahn, 1998). For a *less formal or semi-structured interview*, the interviewer is free to modify the sequence of questions, change the wording, explain them or add to them. In the completely *informal or unstructured interview*, the interviewer may have a number of key issues which she raises in conversational style (Cohen et al., 2007; O'Leary, 2005). This kind of interview is likely to provide greater depth of response (Best & Kahn, 1998).

Lincoln and Guba (1985) suggest that the structured interview is useful when the researcher is aware of what she does not know and therefore is in a position to frame questions that will supply the knowledge required, whereas the unstructured interview is useful when the researcher is not aware of what she does not know, and therefore, relies on the respondents to tell her. The unstructured interview is the most common in qualitative research where the primary intent is to discover what people think and how the views of one individual compare with those of another (Fraenkel & Wallen, 1993).

The interviewing also requires the interviewer to establish a relationship with the interview participant (Mutch, 2005). The interviewer will have given them the research information sheet and consent form before the interview (Cohen et al. 2000; McMillan & Schumacher, 1993). This may involve just a brief discussion at the start to put the participants at ease. The interviewer needs to remind them of the purpose of the research, reassure them of the confidentiality of their contributions, and explain the

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approach used in interviewing (Cohen et al., 2007; Mutch, 2005). In addition, the interviewer needs to establish an appropriate atmosphere such that the participant can feel secure to talk freely (Best & Kahn, 1998; Drew, 1980; Hinds, 2000).

Interview can be taken with either individuals or groups. The group interview requires skillful chairing and attention to the physical layout of the room, so that everyone can see everyone else. Group size is also an issue; too few and it can put pressure on individuals, too large and the group fragments and loses focus (Cohen et al., 2007). Lewis (1992) suggests that group of around six or seven is an optimum size, though it can be smaller for younger children. Therefore, group interview can generate a wider range of responses than individual interview (Cohen et al., 2007). It is often quicker than an individual interview and hence is time saving (Mutch, 2005). However, responses of group interview are difficult to code (Mutch, 2005).

As the subject responds to the questions, the interviewer must record the answer (Cohen et al., 2007). Taking notes is one possibility, but much information may be lost (McMillan & Schumacher, 1993). For an interviewer who attempts to take notes it may be difficult to maintain concentration, and only key points will be recorded (Hinds, 2000). This is in contrast to a recorded session where all information is captured on audio tape, making it easier for the researcher to focus on the interview (Hoepfl, 1997). However, the mere presence of a recorder may disrupt the interview and affect the responses, especially if personal questions are asked (McMillan & Schumacher, 1993). Taped answers also require transcription that takes time (Hind, 2000). Using recordings and notes, the interviewer should become aware of problems of bias, such as selective attention. It is important that the responses made by the respondents should be recorded or transcribed as accurately as possible (Hinds, 2000).

Using interviews in the research has advantages over questionnaires in that the reading ability is not a concern, so the researcher can check the respondent's understanding of the questions and once the respondent has agreed, there is a higher chance of successful completion (Mutch, 2005). At the same time, it provides more in-depth information (Cohen et al., 2007). However, the number of respondents is often limited, because interviews are time intensive (Bell, 2005; Moore, 2000). The interview data can easily become biased and misleading if the person who is being

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interviewed is aware of the perspective of the interviewer. Too often interviewees provide information based on what they think the interviewer wants to hear. Therefore, it is critical for the interviewers to make sure the person being interviewed understands the objective of the interviews (Best & Kahn, 1998).

3.6.3 Observations

Observations are a powerful tool for gathering data in the context of a natural scene. They are used for the purpose of description of setting, activities, people, and the meaning of what is observed from the perspective of the participants (Hoepfl, 1997). These enable the researcher to understand the context of program, to see thing that might otherwise be unconsciously missed, to discover things that participants might not freely talk about in an interview situation, to move beyond perception-based data, and to access personal knowledge (Cohen et al., 2007).

Gathering data by observations should be carefully planned (Drew, 1980). Two factors become immediately important in planning the observation process: (1) the setting and (2) the structure exercised on the observations by the investigation itself (Drew, 1980). Both of these factors can be viewed in terms of a single concept, a continuum of structure (Drew, 1980). The setting may be highly structured, semi-structured, or it may be unstructured (Cohen et al., 2007). A *highly structured observation* will be concerned with a schedule of what it is looking for (Cohen et al., 2007), and typically, something like an observation checklist is used (Dominowski, 1980). A *semi-structured observation* will have schedule or checklists to organize the observation but will gather data to illuminate these issues in a far less predetermined or systematic manner (Cohen et al., 2007). An *unstructured observation* will be far less clear on what it is looking for and will, therefore, have to enter a situation and the observer will record whatever occurs in a presumably natural setting before deciding on its significance for the research (Cohen et al., 2007).

In observation, many researchers rely most heavily on the use of field notes (Hoepfl, 1997). They can capture anything from descriptive and formal accounts of space, actors, acts, and events that are much more interpretive narrative accounts that include goals, feelings, and underlying stories (O'Leary, 2005). Observations may be

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recorded systematically using very structured categorization or counting of behaviors such as a behavior checklist or rating scale (Cohen et al., 2007; Drew, 1980). They may be recorded without a specific guide for the observer. These depend on the structure of the setting imposed by the investigation (Drew, 1980). In addition to field notes, researchers may use photographs, audiotape recorders, and videotape recorders as means of accurately capturing a setting (Hoepfl, 1997). It is important to note that installing video cameras might be selective, and if moveable, they might still be highly selective (Cohen et al., 2007).

Though it is possible that all researchers are some form of participant observer since they cannot study the world without being part of it (Cohen et al., 2007), nevertheless, the role of researchers in observation can be classified into complete participant, participant-as-observer, observer-as-participant, and complete observer (Cohen et al., 2007). For the *complete participant*, the researcher may act as a full participant in the situation being studied with a hidden identity (Cohen et al., 2007). For the *participant-as-observer*, the researcher is part of the social life of participants and documents and records what is happening for research purposes (Cohen et al., 2007). The *observer-as-participant*, like the participant-as observer, is known as a researcher to the group and maybe have less extensive contact with the group (Cohen et al., 2007). With the *complete observer*, the participants do not realize that they are being observed since the researcher watches from outside from hidden cameras and recorders or through one-way mirrors (Burns, 2000; Cohen et al., 2007). Sometimes this type of observation is known as non-participant observation (O'Leary, 2005).

Each of the above roles has both advantages and disadvantages. The complete participant is probably most likely to get the truest picture of a group's activities, and the others less so (Frankel & Wallen, 1993), but the ethical question involving covert observation remains (Borge & Gall, 1989). The complete observer is probably least likely to affect the actions of the group being studied, the others more so. The participant-as-observer will have some effect on what the group does since the researcher is actually member of the group being studied. The observer-as-participant may also influence participant's behaviors since the participants know that they are being observed (Frankel & Wallen, 1993).

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3.6.4 Tests of Conceptual Understanding

As data-gathering devices, tests are among the useful tools and an essential part of the experimental and descriptive studies in education (Best & Kahn, 1998; Cohen et al., 2007). Tests are the instrument designed to describe and measure a sample of certain aspects of human behaviour. They may be used to compare the behaviour, quantified by numerical scores (Best & Kahn, 1998).

Testing may be viewed as a systematic sampling of an individual's characteristics or at a given time under specified conditions. The responses of the individual to given problems, tasks, and questions are summarized to yield one or more index values intended to describe the specific characteristics (Bloom, 1963). The systematic nature of testing makes it possible to use test evidence for a variety of comparative purposes (Bloom, 1963). If uniformity of testing procedures and conditions is assured, it is possible to summarize the responses of an individual and compare his response with those of a number of other individuals. The test scores of several individuals can yield an average which may be compared with the distribution of similar scores for an appropriate population of individuals. Normative data make it possible to determine where individuals stand with respect to other individuals who presumably were tested with the same instrument under similar conditions (Bloom, 1963; Cohen et al., 2007; McMillan & Schumacher, 1993). It is also possible to use summaries of test evidence so as to compare the individual with himself. Thus his score on one test may be compared with his score on another test, or his score at one time may be compared with his score at another time to determine change, progress, or development (Bloom, 1963; Cohen et al., 2007; McMillan & Schumacher, 1993). Of course, the way in which the results of tests are to be summarized must depend on the use to which the results are to be put (Bloom, 1963).

Most tests used in schools are the achievement tests (Best & Kahn, 1989). They are particularly helpful in determining individual or group status in academic learning. Achievement test score are used in placing, advancing, or retaining students at particular grade levels. In research, achievement test scores are used frequently in evaluating the influences of courses of study, teachers, teaching methods, and other factors considered to be significant in education practice (Best & Kahn, 1989).

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For the achievement test, content validity is very important. Content validity is critical for the achievement test (Best & Kahn, 1989; Borg & Gall, 1989). It refers to the degree to which the test items actually measures, or are specifically related to, the traits for which the test was designed. It shows how adequately the test samples the universe of knowledge and skills a student is expected to master (Best & Kahn, 1989). Content validity is based on careful examination of course textbooks, syllabi, objectives, and the judgment of subject matter specialist (Best & Kahn, 1989). Therefore, test developer should consider the representativeness of the sample of items. Selecting the test items needs to take account of the notion of fitness for purpose or the outcome to be measured (Borg& Gall, 1989; Dunn, 1986). That is each test items may be designed following the intended outcome such as to test, according Bloom's Hierarchy of thinking, recall knowledge, knowledge comprehension, application, analysis, synthesis, or evaluation (Cohen et al., 2007).

The reliability of the test is also very important (Dunn, 1986) since it shows that a test is reliable to the extent it measures whatever it is measuring consistently. In tests that have high reliability, errors of measurement have been reduced to a minimum (Best & Kahn, 1989). Best and Kahn (1989) suggest one way to reduce the probability of measurement error is to increase the number of items in a test. An example will help to show why this is so is that using the test with just two multiple choice items. Some students with a great deal of knowledge may do poorly because the test did not sample their knowledge; that is, they knew a lot but not the answers to those two items. Other with only a partial understanding of the course content may guess and get the answer right still others may guess and get both wrong or only one right. Thus, a test with only a few items has a great deal of measurement error. If the number of items is increase, a great deal more of the course content will be sampled so guessing will not play such a great role in determining grades, and the measurement error that exist with just two items will be reduced (Best & Kahn, 1989).

Within the domain of testing, a paper and pencil test has commonly used in science education research (Dunn, 1986). Although the construction of a good paper and pencil test is initially time consuming, its ease of administration and marking, coupled with its re-usability, make it a very cheap form of assessment (Bacon, 2003;

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Cohen et al., 2007). Paper and pencil test, however, has limitation. It is not appropriately for the testing of laboratory activities. This is essentially because the test items measure some attribute which could be developed independently of the laboratory, and hence can be considered invalid (Dunn, 1986). However, the paper and pencil test has high reliability (Dunn, 1986; Schuwirth & van der Vleuten, 2003). A multiple-choice test, for example, is the epitome of reliability since it can often be designed to have one unambiguous answer per item (Dunn, 1986). As Dunn (1986) has noted, the reliability of the test is a relative value rather than any absolute measure. Consequently, the multiple-choice test format is well respected among researchers in educational testing (Bacon, 2003). It is a simple format that can be used to assess complex levels of knowledge or the ability to perform certain tasks. In time, it advances in item analysis, the determination of optimal item difficulty, and the development of discrimination indexes (Bacon, 2003).

Especially, a Two-Tier test - a pencil and paper test in a multiple-choice format – has been recommended by science educators as an effective assessment used to diagnose alternative conceptions (Treagust, 1986). Treagust (1986, 2006) has provided useful guidelines for the development of these instruments, specifically for the purpose of identifying alternative conceptions about various concepts. The first tier of the items consists of a content question, while the second tier elicits a reasoning response. The use of a Two-Tier test allows the investigators to not only understand the incorrect ideas, but also explore their reasoning behind these ideas (Tsai & Chou, 2000; Yenilmez, Sungur & Tekkaya, 2006). Moreover, it facilitates assessment of alternative conceptions of a larger sample in a more efficient and relatively straightforward manner (Chen, Lin & Lin, 2002; Voska & Heikkinen, 2000), and has been widely used in science education research (e.g. Chandrasegaran, Treagust & Mocerino, 2007; Griffard & Wandersee, 2001; Schmidt, 1997; Tan, Goh, Chai & Treagust, 2002).

3.6.5 Concept Map as Evaluation Tool

Concept mapping has also been used as evaluation tool in education research (Kinchin, Hay & Adams, 2000; Ruiz-Primo & Shavelson, 1996). It has been proposed

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to measure the structure and organization of an individual's knowledge (Kaya, 2007; Novak, 1990).

The concept map is a form of two-dimensional diagram that represents the relationships among important concepts (Őzman, Demircioğlu & Coll, 2007). The basic element of a concept map is concept words or phrases that are connected together with linking words or phrases to form complete thoughts (Stoddart, Abrams, Gasper & Canaday, 2000). During concept mapping, the learner graphically represents concepts in a hierarchically arranged structure and begins to progressively differentiate among concepts. Progressive differentiation refers o the learning process in which learners differentiate between concepts as they learn more about them. During the process of integrative reconciliation, the learner recognizes relationships between concepts and does not compartmentalize them (Novak, 1990).

Basic to making a concept map for a piece of knowledge is the ability of the mapper to identify and relate its salient concepts to a general, superordinate concept that requires an understanding of what constituents the concept (Wandersee, 1990). Therefore, concept mapping enables an investigator to assess prior knowledge (Brown, 2003; Buntting, Coll & Campbell, 2006; Stoddart et al., 2000), to evaluate conceptual understanding (Brown, 2003; Francisco, Nakhleh, Nurrenbern & Miller, 2002; Ross & Munby, 1991; Williams, 1998), to identify gaps in knowledge and misunderstandings (Buntting et al. 2006; Novak, 2002; Ross & Munby, 1991), and to explore conceptual change (Kaya, 2007; Novak, 2002; Wallace & Mintzes, 1990).

By using concept mapping as assessment, the individual's ability to make connections between concepts is evaluated (Francisco et al., 2002). In other words, this seeks to understand how individuals integrate new learning into existing conceptual framework by making explicit, conscious connections between concepts as a way to integrate information into memory (Kaya, 2007; Novak, 1990). However, a concept map is difficult to score (Yin, Vanides, Ruiz-Primo, Ayala & Shavelson, 2005), takes time, and needs experience (Taricani & Clariana, 2006) because students' products vary greatly. The total number of links constructed by a student is uncertain and the structure of the map is unfixed (Yin et al., 2005). A possible solution to this scoring difficulty is to ask a student to construct a map selecting from predetermined

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linking phases. This has the advantage that the scoring of this map could be easily automated with computers because the number of valid links is bounded (Yin et al., 2005). However, an open-ended concept mapping, where students may use any concepts and linking terms in their maps is the gold standard for capturing students' knowledge structures (Taricani & Clariana, 2006)

3.6.6 Document Analysis

Documents are an important source of data in many areas of investigation (Best & Kahn, 1998). This is because so many variables of interest are regularly recorded (Sproull, 1995). An analysis of documents enables researchers to study human behaviour in an indirect way (Hinds, 2000). A person's or group's conscious and unconscious beliefs, attitudes, values, and ideas are often revealed in the document they produce (Fraenkel & Wallen, 1993).

Such documents can be classified, according to Mutch (2005), as primary documents (coming from the original source) and secondary documents (having been filtered through a summary, interpretation, or second-hand account). On the other hand, McMillan and Schumacher (1993) classify the documents into personal documents and official documents. However, in document analysis, records, reports, letters, autobiographies, diaries, compositions, themes or other academic work, books, periodicals, bulletins or catalogues, syllabi, court decisions, pictures, films, and cartoons may be used as sources of data (Best & Kahn, 1998).

Best and Kahn (1998) suggest that when using documentary sources, one must bear in mind that data appearing in print may be not necessarily trustworthy. Not only is the authenticity of the document important, but the validity of its contents is crucial. It is the researchers' obligation to establish the trustworthiness of all data that they draw from the documentary sources.

There are several purposes that may be served through document analysis (Best & Kahn, 1998). These are (1) to describe prevailing practice or conditions; (2) to discover the relative importance of, or interest in, certain topics or problems; (3) to discover the level of difficulty of presentation in textbook or in other publications; (4) to evaluate bias, prejudice, or propaganda in textbook presentation; (5) to analyze

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types of errors in students' work; (6) to analyze the use of symbols representing persons, political parties or institutions, countries, or points of views; (7) to identify the literary style, concepts, or beliefs of a writer; and (8) to explain the possible causal factors related to some outcome, action, or event. Therefore, document analysis should serve a useful purpose in yielding information helpful in evaluating or explaining social or educational practices.

3.7 Data Collection in This Study

As mentioned in section 3.6, various methods can be used to collect data to answer the research questions. All methods of collection require rigorous and systematic design and execution that include thorough planning, well considered development, effective piloting, weighed modification, deliberate implementation and execution, and appropriate management and analysis.

Survey involving gathering information from individuals using a questionnaire can reach a large number of respondents; generate standardized, quantifiable, empirical data, as well as some qualitative data; and offer confidentiality and anonymity. It can be descriptive or explanatory, involve entire populations or samples of populations, capture a moment or map trends, and can be administered in a number of ways.

Interviewing involves asking respondents a series of open-ended questions. Interviews can range from formal to informal, structured to unstructured, and can be one-on-one or involve groups. They can generate both standardized, quantifiable data, and more in-depth qualitative data.

Observation relies on researchers' ability to gather data through their senses and allows researchers to document actual behavior rather than responses related to behavior. Observation can range from non-participant to participant, and from structured to unstructured. The observation process is sometimes treated casually, but is a method that needs to be treated as rigorously as any other. The process should include planning, observing, recording, reflecting, and authenticating.

Tests are normally used for evaluation proposes. They may be used to describe and measure a sample of certain aspects of human behaviour, or to compare the

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behaviour quantified by numerical scores. Importantly, the test items need to take account of the notion of fitness for purpose or the outcome to be measured. Reliability and validity of the test are a relative value like any measure.

Concept mapping can be used as evaluating tools in education research. The individual's ability to use linking terms to make connections between concepts may reveal the person's prior knowledge and conceptual understanding. Students' map products vary greatly and difficult to score but these maps give more information about students' knowledge structures.

Documents are rich source of useful information for the research project since so many variable sources of interest are regularly recorded. The documents can be analyzed quantitatively using content analysis or qualitatively using thematic analysis. These enable the researcher to study human behavior in an indirect way. Trustworthiness of document analysis also depends on sources of the documents.

It is obvious that no single method can yield all the information required. According to Johnson and Owuegbuzie (2004), these methods can be mixed in order to maximize strengths, minimize the weaknesses of the research, as well as to address much more comprehensive answer to the research questions (Brewer & Hunter, 1989; Corner, 1991; Greene, Caracelli & Graham, 1989; Owuegbuzie & Leach, 2005). Therefore, the researcher employed a mixed-method in this study.

This study, the researcher conducted a mixed-method study with a temporary triangulation design. In this design, the data collected from each method were used to cross-validate, confirm, or corroborate the findings within a single investigation (Creswell, Clark, Guttmann & Hanson, 2003). The data collecting methods used in this study were conceptula tests, concept mapping, document analysis, questionnaire, interview, and observation.

3.7.1 Conceptual Tests

Enzyme Conceptual Achievement Test (ECAT) and Enzyme Laboratory Achievement Test (ELAT) have been designed to assess students' understanding of the enzyme catalytic reaction concepts covering in the lecture and the concept related to the enzyme kinetics laboratory, respectively.

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3.7.1.1 Enzyme Conceptual Achievement Test (ECAT)

ECAT have been designed to assess students' understanding and misunderstanding of the three key concepts of enzyme catalytic reaction: nature of enzymes, how enzymes work, and influence of physical and chemical environment on enzymes' function. This test includes 18 two-tier test items (see Appendix A). In the first tier, the students must indicate whether or not the item statement is correct. In the second tier, they must select the reasons for their answer.

The items for this test have been developed based on the two-tier multiple choice format described by Peterson et al.(1989), and Treagust (1986).The first tier of each item consists of a content question having three choices, the second part of each item contains four possible reasons for the answer given in the first tier. Included in these reasons are the correct answer and three identified alternative reasons involving misconceptions.

For development of ECAT, the test items were based on literature, biochemistry textbooks (Berg et al., 2001; Metzler et al., 2001; Mckee & Mckee, 2003; Smith et al., 1996; Voet et al., 2004), course syllabi (Biochemistry Department, 2006), as well as course objectives especially in the enzyme kinetics topics. An incorrect reason for each item was based on students' alternative views of enzyme catalyzed reaction as well as on chemical kinetics identified from literature and teaching experiences. This test consists of 18 items that are classified into three categories to measure three key concepts of enzyme kinetics: the nature of enzymes, how enzymes work, and the influence of physical and chemical environments on enzymes function.

To assure the content validity and reliability of the test, it was checked by two expert biochemistry teachers and one expert science educator, then, the test was piloted with 253 second year undergraduate science students. The Cronbach's alpha reliability coefficient of the pilot study was 0.62.

In this study, the test was administered on students before (pre-test) and after the laboratory class (post-test 1), and at the end of the lecture class (post-test 2). In addition, this test was administered to students at two months after the lecture class (retention test). The content of these tests was similar but the item sequencing was

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different. The results of pre-test, post-test, and retention test were compared to evaluate the students' learning achievement.

3.7.1.2 Enzyme Laboratory Achievement Test (ELAT)

The open-ended questions were designed to assess students' understanding of the concepts related to the enzyme kinetics laboratory (see Appendix B) such as measuring rate of enzyme reaction, as well as to assess students' gain in laboratory skills such as designing the control experiment, and generating a conclusion for the experiment based on their empirical data.

For constructing of ELAT, the researcher examined the students' laboratory manual and considered the learning objectives of an enzyme kinetics laboratory unit. The contents of this test had been checked by two expert biochemistry teachers. This test was piloted with 196 second year undergraduate science students. The Cronbach's alpha reliability coefficient of the pilot study was 0.63.

ELAT was administered to students before (pre-lab test) and after the laboratory session (post-lab test). The results of pre- and post-tests were compared to evaluate the laboratory learning outcomes.

3.7.2 Student Concept Maps

The concept maps of enzyme catalyzed reaction written by students were collected before and after the laboratory class, as well as at the end of the lecture class. These concept maps were used to assess prior student knowledge, and to determine the progression of student's knowledge of enzyme kinetics. These results were compared with the achievement test scores for corroboration.

To evaluate student's knowledge about enzyme kinetics, the quality of the links in the concept map was considered. The quality of links has been shown to be an important indicator of understanding (Kinchin, 2000) because it requires the mapper to actively select appropriate linking words (Bunting et al., 2006; Őzman et al., 2007).

However, students' limited practice of constructing concept maps may result in limited representation of their conceptual understanding (Kaya, 2007). Therefore in this study, before constructing maps, students were given an example of concept map

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of another topic (see Appendix C) and were briefed focussing on using the linking terms or words to link between concepts. In addition, the data from other measures such as ECAT, ELAT, and students documents were used to triangulate with those of student concept maps.

3.7.3 Document Analysis

Two major sources of documents were considered in this study: students' documents and teacher' documents. These documents were analyzed using a thematic approach to make valid inferences from the text in order to provide in-depth information as well as to be used for triangulation with other collection methods.

3.7.3.1 Students' Documents

Students' posters, their laboratory reports, and their answers on post-laboratory exercises (see Appendix D) were collected to provide in-depth details about student understanding of enzyme kinetics. In addition these documents were used to assess student abilities such as designing the experiment and interpretation and discussion of their results. The analysis of these documents, therefore, focussed on the methods they used in conducting the experiment, conclusion of the results, and discussion of their findings.

Since the laboratory report is constructed away from the laboratory, the results may be copied from the literature or fellow students or even adjusted to give an acceptable value (Dunn, 1986), one copy of the results and their experimental design was collected to the supervisor at the end of the laboratory session, and the other retained by the student.

Such data from the analysis of these documents were used to triangulate with those data form ECAT, ELAT, and students' concept maps.

3.7.3.2 Teachers' Documents

Teachers' documents such as teaching outline, laboratory manual, and power point were collected to provide information about the concepts of enzyme kinetics that teacher introduced to students. This information was used to analyze for students'

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understanding of enzyme kinetics. This data was used to corroborate the data from conceptual test, concept map, as well as student's documents.

In addition, the teacher journal notes of events in the laboratory (such as data management, cooperative learning, question and students' feedback in the practical activities) were also collected to discover how students learned in the laboratory class. This data was used to corroborate the researcher observation data.

3.7.4 Questionnaires

Two questionnaires were used in this study. One was used to gather information regarding student preferences and perceptions of the learning environment. The other was used to gain information regarding teachers' views of the learning approach.

3.7.4.1 Constructivist Learning Environment Survey (CLES)

This instructional design was based on constructivist epistemology. Constructivist Learning Environment Survey (CLES) designed by the Salish I Research Project (1997) was adapted and translated from English to Thai and then used to measure the extent to which students perceived their learning environments as consistent with a constructivist epistemology. The information regarding students' perceptions of science learning environment in response to the learning cycle approach was obtained.

The items of CLES questionnaire of Salish I Research Project version were adapted from those of Taylor, Fraser, and White (Salish I Research Project, 1997). In Salish I Research Project version, CLES questionnaire contains 42 items, which students must answer by selecting one of five responses: almost always, often, sometimes, seldom, and almost never. These items are classified into six scales (seven items in each of six scales) relevant to an epistemological constructivist learning environment that are personal relevance, scientific uncertainty, critical voice, shared control, student negotiation, and attitude (Salish I Research Project, 1997).

Personal Relevance (PR) scale focus on the connection of science to students' out-of-school experiences, and on making use of students' everyday experiences as a

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meaningful context for the development of students' scientific knowledge (Salish I Research Project, 1997).

Scientific Uncertainty (SU) scale assesses the extent to which opportunities are provided for students to experience scientific knowledge as arising from theory-dependent inquiry, involving human experience and values, evolving and non-foundational, and culturally and socially determined (Salish I Research Project, 1997).

Critical Voice (CV) scale examines the extent to which a social climate has been established in which students feel that it is legitimate and beneficial to question the teacher's pedagogical and methods and to express concerns about any impediments to their learning (Salish I Research Project, 1997).

Shared Control (SC) scale is concerned with students being invited to share the teacher control of the learning environment, including the articulation of learning goals, the design and management of learning activities, and the determination and application of assessment criteria.

Student Negotiation (SN) scale assesses the extent to which opportunities exist for students to explain and justify to other students their newly developing ideas, and to listen to and reflect on the viability of other students' ideas.

Attitude (AT) scale emphasizes students' participation in the activities, their sense of the worthwhileness of the activities, and the impact of the activities on student interest, enjoyment and understanding (Salish I Research Project, 1997).

The CLES questionnaire has been used as source of both predictor (such as predicting the relationship between classroom environment and students' achievement) and criterion variables in a variety of research studies (e.g. Nix, Fraser & Ledbetter, 2005; Tsai, 2000; Wolf & Fraser, 2008). This questionnaire has been validated by many researchers. For example, in Western Australia, Taylor, Fraser & Fisher (1997) established the validity and reliability of the CLES with a sample of 494 12-year-old students in 41 science classes in 13 schools. Four of the CLES scales that are Personal Relevance, Critical Voice, Shared Control, and Student Negotiation had alpha reliability above 0.80, while the alpha reliability of Uncertainty scale was lower than this.

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The CLES also has been validated for use in Korea (Kim, Fisher & Fraser, 1999). Kim et al. (1999) translated the CLES into the Korean language and administered it to 1,083 science students in 24 classes in 12 schools. The CLES Korean-language version was replicated for both actual and a preferred form of CLES. The alpha reliability ranged from 0.64 to 0.87 for actual form and from 0.79 to 0.91 for preferred form.

Also Aldridge, Fraser, Taylor, and Chen (2000) and Aldridge and Fraser (2000) established cross-validation of the CLES. The CLES has been translated into Chinese for use in Taiwan. In this cross-national study, the original English version was administered on 1,081 science students in 50 classes in Australia, while the new Chinese version was administered to 1,879 science students in 50 classes in Taiwan. The scale reliabilities for the CLES in the two countries were similar (the scale reliability estimates ranged from 0.87 to 0.97 in Australia and from 0.79 to 0.98 in Taiwan). These results indicate that CLES questionnaire displayed good validity and internal consistency reliability.

In this study, CLES questionnaire was translated into Thai language by the researcher, and then this translation was checked the validity by the expert in English language education. This questionnaire was first piloted before being administered to the sample of this study with 57 forth year undergraduate science students. The Cronbach's alpha reliability coefficients of this questionnaire for pilot study were 0.84 and 0.88 for pre- and post-questionnaires respectively. This CLES Thai version was also piloted with 84 undergraduate science students. The results were similar to the first pilot study (the alpha reliability were 0.83 and 0.88 for pre- and post-questionnaires respectively. The internal reliability of each scale of CLES were 0.59 for PR scale, 0.66 for SU scale, 0.65 for CV scale, 0.66 for SC scale, 0.86 for SN scale, and 0.71 for AT scale. For CLES Thai version, it was necessary to modify some items of the original English version. For example "I learn about the world *outside of school*" was changed to "I learn about the world *in relevance to my life*".

The CLES Thai-language version questionnaire (see appendix E) is administered on seventy two undergraduate students who participated in this research before (pre-CLES questionnaire) and after finishing the instruction (post-CLES

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questionnaire). The pre-CLES questionnaire is used to focus on student perceptions and preferences on the learning environment in the traditional class, while the post-CLES questionnaire is used to focus on their perceptions and preferences on the learning environment in the learning cycle class. The item statements of both questionnaires are same, but at the end of the post-questionnaire open-ended questions are posted to give students an opportunity to write their opinions of the learning activities that they had experienced. The results of both questionnaires are compared to evaluate the effectiveness of the learning cycle approach used in this study.

3.7.4.2 Teacher's Perception toward Learning Environment Questionnaire (TPLEQ)

To obtain information regarding teacher's views of the effectiveness of this learning approach, a Teacher's Perception of Learning Environment Questionnaire (TPLEQ) was administered to the teachers at the end of instruction.

TPLEQ was adapted from Lavoie's (1999) questionnaire. The original questionnaire had been used to examine the effects of adding a prediction/discussion phase to the learning cycle prior to the exploration. The item statements of this questionnaire required the teachers to compare the effectiveness of this strategy with the original learning cycle. Some items focused on the effect of the prediction. These did not fit to the purpose of this study. Therefore, the researcher modified the statements and questions of the original version to fit with this study, and also added some questions that may useful for implementation.

TPLEQ consists of 13 item statements that required the teachers to compare the effectiveness of the learning cycle approach used in this study with the traditional taught in biochemistry class. This was a Likert-scale questionnaire which the teachers must answer using one of five responses: strongly agree, agree, neutral, disagree, and strongly disagree. This questionnaire also posed a series of short-answer questions to allow the teachers write their opinions of this learning strategy such as the positives and negatives of this learning strategy and tips for its improvement. This questionnaire had been checked by two teachers involved in enzyme laboratory unit to ensure that they understood the statements and questions used in this questionnaire.

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3.7.5 Interviews

Using interviews, participants were given a chance for their voices to be heard. The semi-structured interview was used in this study. The questions used in the interviews (see Appendix G) were related to those of questionnaire, the interviews seek to discover how instructional designs were working, and the overall picture of difficulties or disappointments students had experienced. These questions were piloted before used to assure that the students understand them.

The semi-structured interview had advantages. Rather than having a specific interview schedule or none at all, an interview guide may be developed for some parts of the study in which, without fixed wording or fixed ordering of questions, a direction was given to the interview so that the content focused on the crucial issues of the study (Cohen et al., 2007; O'Leary, 2005). This permited greater flexibility than the close-ended type and permited a more valid response from the perspective of participants since the interviewer can probe more deeply using open-form questions to obtain additional information (Best & Kahn, 1998; Burns, 2000).

In this study, a stratified sample of participants based on student groups in a laboratory class was selected for interviews at the end of the instruction. This is because five teachers act as facilitators to the groups of students during their practical work that may affect the outcome of this study. A stratified sample was used in order to get more accurate representation of the population (Best & Kahn, 1998). To attain this representation, the students participated in this study were divided into several groups based on their groups in a laboratory work, and then a random sample from each group were selected for in interviewed.

Audiotape recorder was used to record the conversation between interviewer and participant. Recording interviews on tape was preferred because this is convenient and obviated the necessity of writing during the interview, which may be distracting to both interviewer and subject. The tone of voice and emotional impact of the response were preserved by the tapes (Best & Kahn, 1998) that may be replayed as often as necessary for complete and objective analysis at a later time.

The findings from interviews were used corroboratively with the data from the other sources such as questionnaire or classroom observation.

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3.7.6 Classroom Observation

The unstructured observation was used to investigate how students learned and how teachers taught. In the observations in this study, the role of the researcher was as participant-as-observer as described in section 3.6.1. The participant-as-observer was very useful because by staying in a situation, the researcher was able to get more details of behavior or organizations (Bell, 2005). The researcher was able to have direct contact or systematically share with participants in so far as circumstances permit. This enabled the researcher to obtain the participants' account of their actions (Nandhakuma & Jones, 1997). This also facilitated the generation of thick descriptions, particularly of social process and interaction, which let the researcher to accurate explanations and interpretations of events (Cohen et al., 2007).

However, the researcher recognized that the observer may become emotionally involved and lose perspective during an observation (Borg & Gall, 1989; Cohen et al., 2007). Therefore, the observation data in this study were validated by comparison with those of the teachers' journal notes. In addition, the observations were recorded by audiotape-recorders and video-cameras. Audiotape-recorders were used to record student-student and student-teacher conversations in the groups during laboratory activities. Video-cameras were used to capture students' activities and interactions during laboratory class. These observation data were used for corroboration with other data collection methods, and also for providing specific examples of the events or processes identified in the teachers' journal notes. These multiple data sources allow for triangulation to increase trustworthiness of the results (Mathison, 1988).

The processes for collecting data used in this research study are summarized in Table 3-2.

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Table 3-2 The process for collecting data in this study

Time	Type of data	Data collecting tools
Before laboratory class	Student perceptions and preferences of traditional learning approach	Pre-CLES questionnaire
Beginning of laboratory activities	Students' prior knowledge	ECAT (pre-test) ELAT (per-lab test) Concept Mapping (Map1)
During laboratory activities	Student activities Student-student interaction Student-teacher interaction	Unstructured observation - Audio-recorders - Video cameras Laboratory manual
End of laboratory class	Students' understanding of enzyme kinetics and the other learning outcomes	ECAT (post-test1) ELAT (post-test2) Concept mapping (Map2) Students' posters Teachers' journal notes
During the lecture class	Which concepts are introduced to students	Unstructured observation - Video cameras Teacher documents (PowerPoint)
End of lecture class	Student understanding of enzyme kinetics and the other learning outcomes	ECAT (post-test2) Concept Mapping (Map3)
	Student perceptions of and preferences in this learning environment	Post-CLES questionnaire Semi-structured interviews
	Teacher perception of this learning approach	TPLEQ
After lecture class	Students' understanding of enzyme kinetics and the other learning outcomes	Laboratory reports Post-laboratory exercises
Two months after lecture class	Students' understanding of enzyme kinetics	ECAT (retention test)

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3.8 Data Analysis

Once the data were collected, it was the researcher's task to analyze them, drew certain conclusions concerning the subjects' performance, and interpreted the data in terms of the original research question. The role of data analysis was to bring data together in a meaningful way and enabled researchers to interpret or make sense of it (Wilkinson, 2000).

The data collected in this study were analyzed by both quantitative and qualitative analyses based on the data type. However, the results of these analyses were put together in order to draw conclusions to answer the research questions.

Here, the approaches used to analyzed data obtained from each instrument are described.

3.8.1 Analysis of Conceptual Tests

3.8.1.1 Analysis of ECAT (Enzyme Conceptual Achievement Test)

The two-tier questions needed such data analysis categories because the responses for the first tier questions comprised the knowledge domain, whereas the other tier related to understanding (Çalik, Ayas & Coll, 2005). The use of such classification criteria for student responses provided the researchers with an opportunity to compare students' understanding.

In this study, the students' responses to ECAT were analyzed by using the following categorization modified from Çalik et al. (2005). A correct choice with the correct reason (3 points), correct reason with or without incorrect choice (2 points), correct choice with or without incorrect reason (1 points), and incorrect choice with or without an incorrect reason (0 point). However, the researcher did not determine the criteria before analyzing and administering procedures. That was the students' responses to ECAT were firstly examined, and the criteria merged from these data. To validate the scoring, the students' responses were analyzed by the researcher and three expert biochemistry teachers, separately.

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The student's average score for each test (pre-test, post-test 1 & 2, and delayed post-test) were compared to discover the progress of students' understanding of enzyme kinetics.

Besides that the student's responses on each item were classified into three levels of understandings, i.e., non-understanding, partial understanding, and understanding. Then the responses on each item were grouped according to three key concepts of enzyme kinetics topic to be measured. These three key concepts were the nature of enzyme, how enzyme work, and the influences of physical and chemical environments on enzymes' function. The results from pre-test, post-test (1 & 2), and delayed post-test for each concept were compared to discover students' conceptual understanding on enzyme kinetics.

3.8.1.2 Analysis of ELAT (Enzyme Laboratory Achievement Test)

Since ELAT consists of open-ended questions, the teacher and the researcher discussed and constructed criteria for scoring the student answers. The probably answers for each item with the given points were pre-determined and validated by two experts in biochemistry, and used as criteria for scoring (see Appendix B). Then the answers were scored by the teacher.

The results from pre-lab and post-lab test were compared using paired *t-test* statistics analysis. This allowed the researcher to evaluate the effectiveness of the laboratory work on students' understanding of enzyme kinetics.

3.8.2 Analysis of Students' Concept Maps

In many research studies, the scoring of an open-ended concept map components has been undertaken quantitatively, based on the scoring protocol devised by Novak and Gowin (1984). This tends toward an aggregate score of factors including the number of valid links presented, the degree of cross-linkage indicated, the amount of branching, the hierarchical structure, and examples (Kaya, 2007; Kinchin, Hey & Adams, 2000; Ruiz-Primo & Shavelson, 1996). This has sometimes been done in comparison with an expert map (Kinchin et al., 2000; Ruiz-Primo & Shavelson, 1996).

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Kinchin et al.(2000) have argued that the scoring of only valid links (corrected links) also misses the point that invalid links may have a value to the student by supporting more valid links and so to contributing to the overall knowledge structure that student is using as a basis for further learning. The invalid links in a student's concept map may reveal much about the thought process that lead a student along a particular path of understanding. They note that, as invalid links are seen as being of equal importance to valid links (in term of awareness), the time-consuming process of assessing the validity of links is avoided. Furthermore, they suggest a qualitative evaluation of students' understanding by a concept map needs to consider the scheme differentiates maps in terms of their complexity, resilience in accommodation additions, the establishment of a context for the key concepts, degree of appreciation of a wider viewpoint, and its relationship with the expert view.

Although several researchers suggested that the scoring of concept map should consider the structure complexity of the map (Kinchin et al., 2000; Yin et al., 2005), this complexity was not mentioned in this study because the participants of this study were not familiar with writing the concept map for a long period so that they may not be able to write a more complex map.

In this study, each student's map was scored by the researcher and one teaching assistant. The students' map was scored by counting only the linking terms. The linking terms in one map were counted two times. First, all linking terms presented in each map were counted (total linking terms). Second, only the correct linking terms were counted (total accuracy link). Next, this map was compared with an expert map (this map was constructed by the researcher then validated by the expert in biochemistry, see the expert map in Appendix I). By comparison with the expert map, the number of linking terms mentioned by the students is expressed as a proportion of terms in the expert concept map. This proportion was scaled, according to Yin et al. (2005), from complete (100%) to substantial (67% to 99%), to partial (33% to 66%), to small (1% to 32%), to none (no linking words mentioned or irrelevant links mentioned).

Counting both accuracy link and total link of each map allowed the researcher to see the amount of accuracy and invalid links at the same time. In addition, the

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comparison with the expert map allowed the researcher to see the dispersion of the level of students' understanding.

The results of pre- and post-concept maps were compared in order to evaluate the progress of student's understanding.

3.8.3 Analysis of Documents

The documents can be analyzed by either content analysis or thematic approach (Mutch, 2005).

Most *content analyses* in education have been aimed at answering questions directly relating to the document analyzed. They have been generally concerned with fairly simple classifications or tabulations of specific information. They usually produce quantitative results in the form of numerical frequencies. Content fitting the various categories covered by the analysis is usually tallied in terms of frequency of occurrence (Borg, 1963).

The *thematic approach* seeks to analyze the text with an open mind and tries to allow it to "speak for itself". Thematic analysis of documents tends to answer the questions such as What are text's key messages? How are words used? and What important themes emerge? (Mutch, 2005). This approach often aims at gaining new insights into complex social and psychological variables. It is much more difficult to carry out than the simple frequency study (Borge, 1963).

This research study used a thematic approach to analyze both student's and teacher's documents.

3.8.3.1 Analysis of Student's Documents

Students' laboratory work sheets, their laboratory reports, their post-laboratory assignments, and their posters were analyzed using a thematic approach to discover students' conceptual understanding of enzyme kinetics and to seek their abilities to design experiment, to interpret the results, to draw a conclusion, and to communicate their findings. These results were also used corroboratively with the other methods such as tests and concept map.

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3.7.3.2 Analysis of Teacher Documents

A thematic approach was used to analyze the teacher documents such as teaching plan, laboratory manual, and power point that the teacher used in teaching of enzyme kinetics topic. The analyses of these documents seek to discover what concepts of enzyme kinetics that teacher introduced to students and to understand how the concepts were introduced to them. These results were used corroboratively with students' conceptual understanding found form the other methods such as tests and student's documents.

The teacher journal notes of events in the laboratory were also analyzed by using thematic approach to discover what were going on in the class or how the students learned. This data was use corroboratively with the observation data.

3.8.4 Analysis of Questionnaire Data

3.8.4.1 Analysis of CLES questionnaire

Before analysis of students' responses on the CLES questionnaire, their responses on a Likert scale of each item statements were coded with the numbers. Since the CLES questionnaire consists of both positive and negative statements, coding was different. For positive item statements, the "almost always" choice would receive a 5, moving to the "almost never" choice which would receive 1. For negative item statements, the numbering procedure was reversed (see Appendix F).

Then, mean scores and percentage of response in each scale of the CLES was analyzed to reveal their perceptions of the learning environment. The results of pre-CLES (focuses on students' perception of the traditional learning approach) and post-CLES (focuses on students' perception of the inquiry-based learning cycle) were compared using paired *t-test* statistics analysis to discover how the inquiry-based learning cycle influenced students' perception toward science learning according to the constructivist epistemology.

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3.8.4.2 Analysis of TPLEQ

Before analysis of the teachers' responses on the TPLEQ, their responses on a Likert scale of five responses: strongly agree, agree, neutral, disagree, and strongly disagree were coded with numbers from 5 to 1.

The number of teachers' responses on a Likert scale of TPLEQ for each item were counted and analyzed to discover the overall teacher views about the effectiveness of the learning cycle approach. In addition, the teacher's responses to short answers question were summarized to be used for the implementation for teaching and learning.

3.8.5 Analysis of Interview Data

The conversations between the interviewers and the respondents were transcribed, then they were analyzed by using thematic approach to discover how the learning cycle were working and an overall picture of what difficulties or disappointments students had experienced.

The results of the interviews were used corroborate the other methods such as questionnaires and observations.

3.8.6 Analysis of Observation Data

The data from videotape and audiotape-recorders during the laboratory period were analyzed using a thematic approach where theme will be emerged from data. The analyses of these data aimed to discover what were going on in the class or how the teacher taught and how the students learned. The data from each source including the teacher journal notes were also put back together in new ways by making connection between a category and its subcategories. These results were compared to seek confirming or disconfirming information.

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CHAPTER IV
RESULTS

Overview

This chapter presents the main findings of the research. It is divided into two parts: scientific part and educational part, and then are summarized at the end of the chapter.

4.1 Scientific Part

The research findings in scientific part are regarding the development of a simple detection for bromoperoxidase activity in red seaweed by using bromophenol red as organic substrate. This new substrate gave the colored products which can be followed visually and/or colorimetrically. The benefits of using bromophenol red for assay of bromoperxidase activity compared with that of phenol red, one of the well known substrate, was presented. The kinetics of red seaweed bromoperoxidase was revealed.

4.1.1 Specific Activity of Crude Bromoperoxidase

Crude extract from red seaweeds *Gracilaria tangi* prepared as described in section 3.3.1.1 and 3.3.1.2 contained 0.19 mg protein per gram wet weight of seaweeds. The specific activity was found to be 0.1 U/mg protein.

4.1.2 Bromoperoxidase Assay Using Br_2PR as Organic Substrate in Comparison with PR

When bromoperoxidase activity was assayed by using 4.17 μM phenol red (PR) as substrate in the reaction mixture containing 20 mM KBr, and 0.2 mM H_2O_2 in 0.1 M phosphate buffer pH 5.5, the yellow color of PR changed to blue. The absorption spectra of the reaction mixture in Figure 4-1 show the decrease in PR at

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wavelength about 430 nm. The PR was converted to blue color product, with an increasing in absorption at 590 nm. When bromophenol red (Br_2PR) was used as substrate under similar condition, the pink color substrate changed to blue color product which was measured from a decrease in absorption at 430 nm and an increase in absorption at 590 nm (Figure 4-2). Br_2PR was converted to blue color product within 5 min while PR was converted to blue product in longer time, i.e., in 10 min. The increase in absorption spectra of blue color product was higher for Br_2PR than that for PR

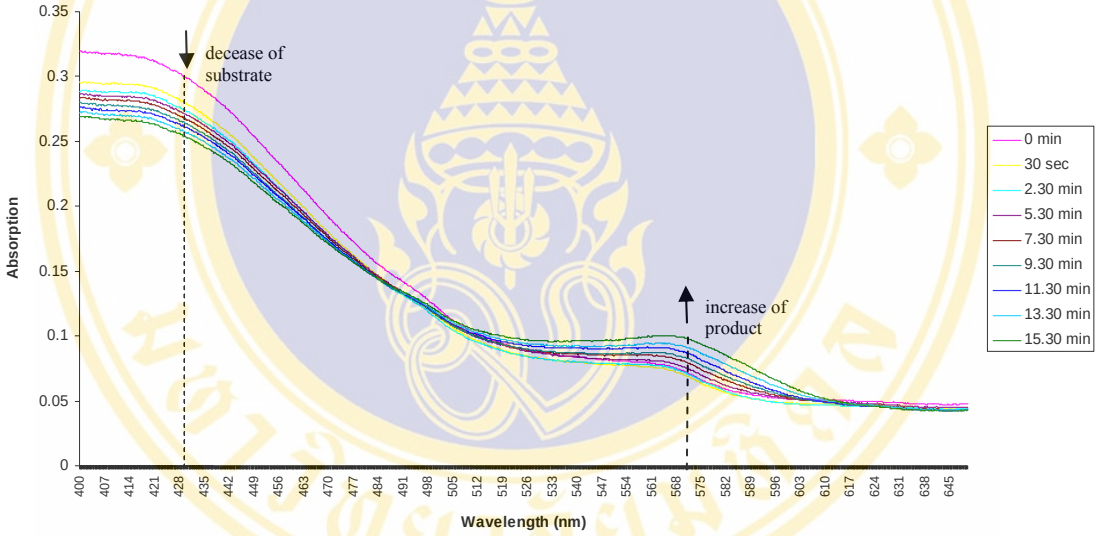

Figure 4-1 Absorption spectra of the reaction mixture for assay of bromoperoxidase using PR as organic substrate. The reaction mixture contained 4.17 μM PR, 20 mM KBr, and 0.2 mM H_2O_2 in 0.1M potassium phosphate buffer pH 5.5 and crude bromoperoxidase (0.1 U/mg protein). The absorption spectra were recorded by using Shimadzu UV-visible recording spectrophotometer at 25°C.

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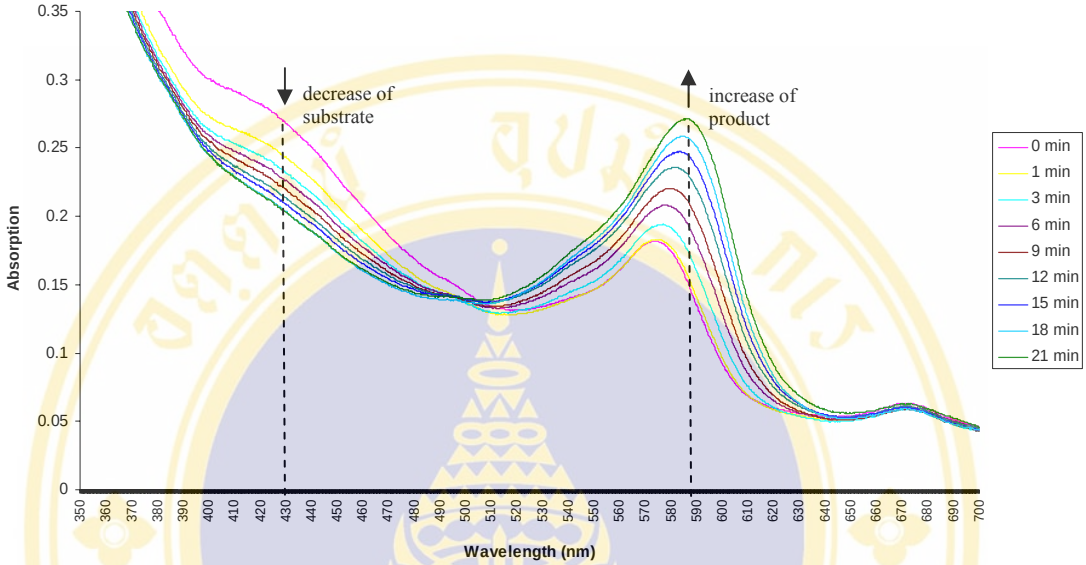

Figure 4-2 Absorption spectra of the reaction mixture for assay of bromoperoxidase using Br_2PR as organic substrate. The reaction mixture contained 4.17 µM Br_2PR, 20 mM KBr, and 0.2 mM H_2O_2 in 0.1M potassium phosphate buffer pH 5.5 and crude bromoperoxidase (0.1 U/mg protein). The absorption spectra were recorded by using Shimadzu UV-visible recording spectrophotometer at 25°C.

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4.1.3 Red Seaweed Bromoperoxidase Assay by Using Br_2PR as Substrate

Upon bromoperoxidase-catalyzed bromination of BR_2PR (at 33.33 μM) in the presence of 20 mM KBr, and 1 mM H_2O_2 in 0.1 M acetate buffer pH 6.5, the pink color substrate was converted to blue color product; the intensity of blue color increased with time (Figure 4-3a). The absorption at 590 nm increased from 0 to 40 min and reached plateau after 40 min (Figure 4-3b). The initial rate of bromoperoxidase as measured by following the increase of blue product was found to be 0.03 OD_{590}/min.

Figure 4-3a The color product of the reaction catalyzed by red seaweed bromoperoxidase. The reaction mixture in total volume 3 ml contained 33.33 μM BR_2PR, 20 mM KBr, and 1 mM H_2O_2 in 0.1 M acetate buffer pH 6.5 and 0.5 ml crude enzyme (0.1 mg protein, 0.1 U/mg protein).

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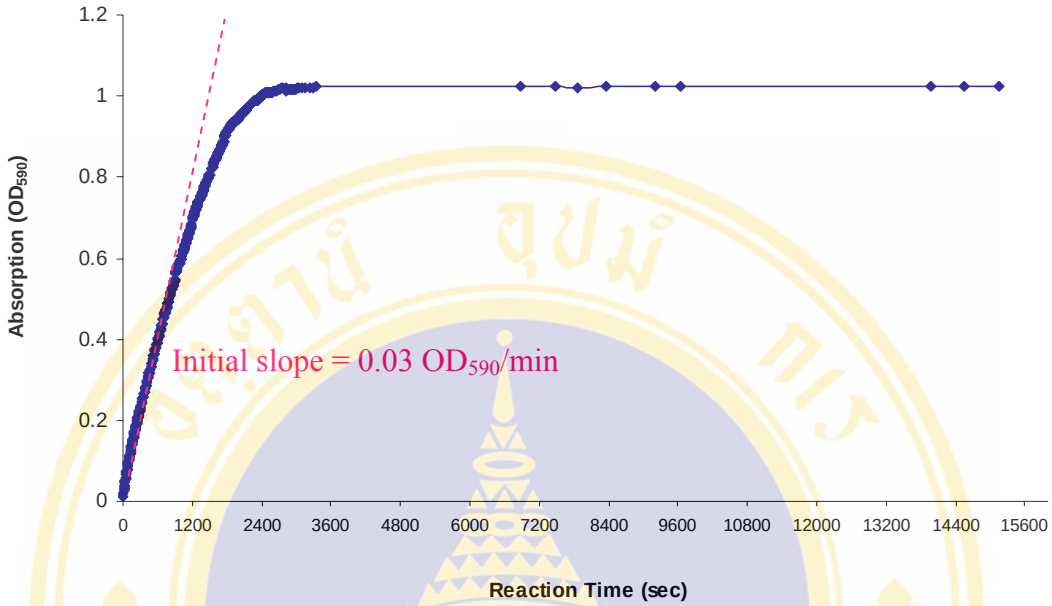

Figure 4-3b Absorption at 590 nm versus time of the reaction mixture for bromoperoxidase-catalyzed bromination of BR_2PR. The reaction mixture contained 33.33 µM BR_2PR, 20 mM KBr, and 1 mM H_2O_2 in 0.1 M acetate buffer pH 6.5 and 0.5 ml crude enzyme (0.1 mg protein, 0.1 U/mg proteins) in total volume 3 ml.

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4.1.4 pH Optimum for Red Seaweed Bromoperoxidase

The activity of red seaweed bromoperoxidase changed with pH (Figure 4-4). The rate in catalyzing brominating reaction increased with increasing pH from 5.5 to 6.0 to 6.5 and decreased as pH increase from 6.5 to 7.0. The red seaweed bromoperoxidase showed highest activity at pH 6.5.

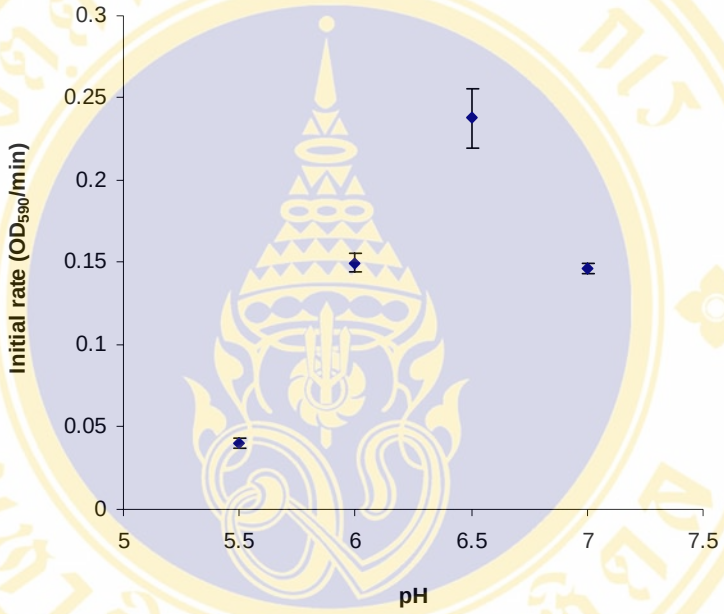

Figure 4-4 Red seaweed bromoperoxidase activity at various pH. Crude enzyme was assayed for its activity in the presence of 20 mM KBr, 12.5 µM Br_2PR, and 0.2 mM H_2O_2 in 0.1 M acetate buffer (pH 5.0, 6.0, 7.0 and 7.5) at 25°C. Error bars in the graph show standard deviation of a triplicate results.

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4.1.5 Effects of Enzyme Concentrations on Red Seaweed Bromoperoxidase Activity

In the presence of 20 mM KBr, 33.33 μM Br_2PR, and 0.2 mM H_2O_2 in 0.1 M acetate buffer pH 6.5, the activity of red seaweed bromoperoxidase increased with increasing concentration of crude enzyme (0.1 U/ mg protein) from dilution 1/20 (0.01 mg protein) to 1/5 (0.04 mg protein). After a dilution 1/5 of crude enzyme, increase in enzyme concentration did not increase the enzyme activity (Figure 4-5).

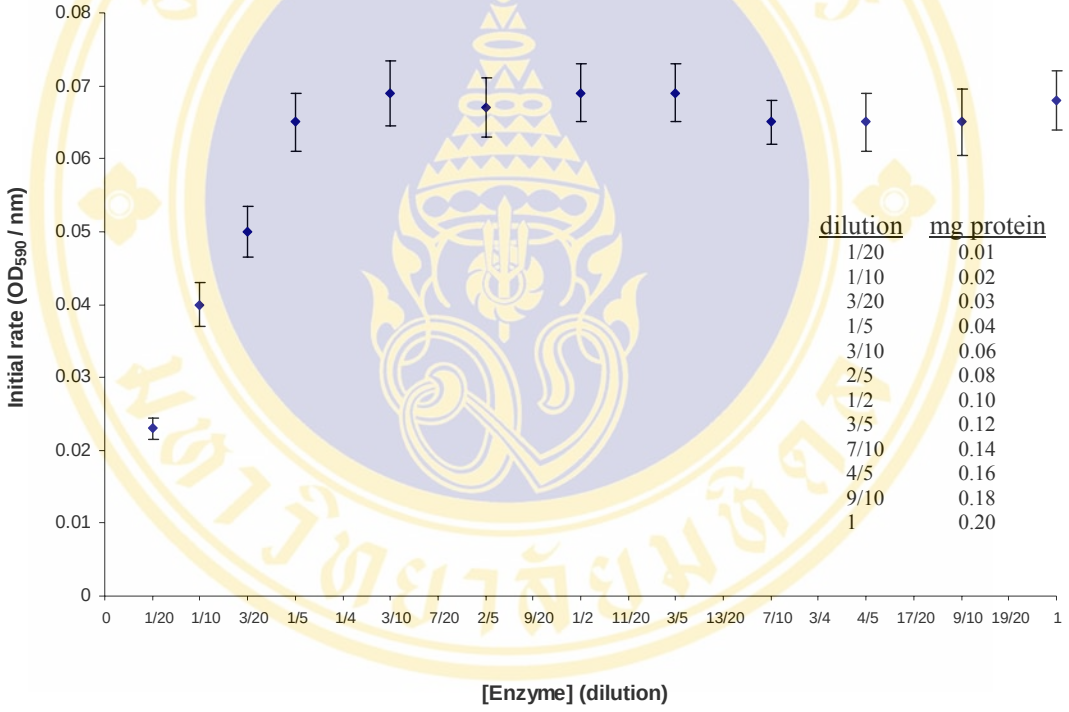

Figure 4-5 The plot of initial rate vs. concentration of crude bromoperoxidase (0.2 mg protein/ml, 0.1 U/mg protein) in the reaction mixture containing 20 mM KBr, 33.33 μM Br_2PR, and 0.2 mM H_2O_2 in 0.1 M acetate buffer pH 6.5 at 25°C. Error bars in the graph show standard deviation of a triplicate results.

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4.1.6 Effects of Br_2PR Concentrations on Red Seaweed Bromoperoxidase Activity

The red seaweed bromoperoxidase activity increased as the concentration Br_2PR increased from 4.17 to 54.17 μM (Figure 4-6a). V_{max} (the velocity that all enzyme-active sites are filled with substrate, Br_2PR) of this enzyme-catalyzed reaction was not obtained from Michaelis-Menten plot. However, V_{max} of 0.166 OD_{590}/min was obtained from Lineweaver-Burk plot (Figure 4-6b). The K_m value (the Michaelis constant which equals to the substrate concentration that gives half of the maximal velocity) was obtained from Lineweaver-Burk plot. K_m for Br_2PR was found to be 31 μM.

4.1.7 Effects of KBr Concentrations on Red Seaweed Bromoperoxidase Activity

The red seaweed bromoperoxidase activity increased as concentration KBr increased from 0.07 to 1.67 mM (Figure 4-7a). The velocity that all enzyme-active sites are filled with KBr (V_{max}) was not obtained from Michaelis-Menten plot. The V_{max} obtained from Lineweaver-Burk plot (Figure 4-7b) was found to be 0.025 OD_{590}/min. The Michaelis constant (K_m) obtained from Lineweaver-Burk plot for KBr was 0.33 mM.

4.1.8 Effects of H_2O_2 Concentrations on Red Seaweed Bromoperoxidase Activity

In the presence of 20 mM KBr, 33.33 μM Br_2PR in 0.1 M acetate buffer pH 6.5, the activity of seaweed bromoperoxidase increased as concentration of H_2O_2 increased from 0.1 to 0.5 mM. However, at more than 0.5 mM H_2O_2, the increase in the concentration of H_2O_2 caused a decrease in the enzyme activity (Figure 4-8a). V_{max} and K_m for H_2O_2 obtained from Lineweaver-Burk plot (Figure 4-8b) were 0.1 OD_{590}/min and 0.15 mM, respectively.

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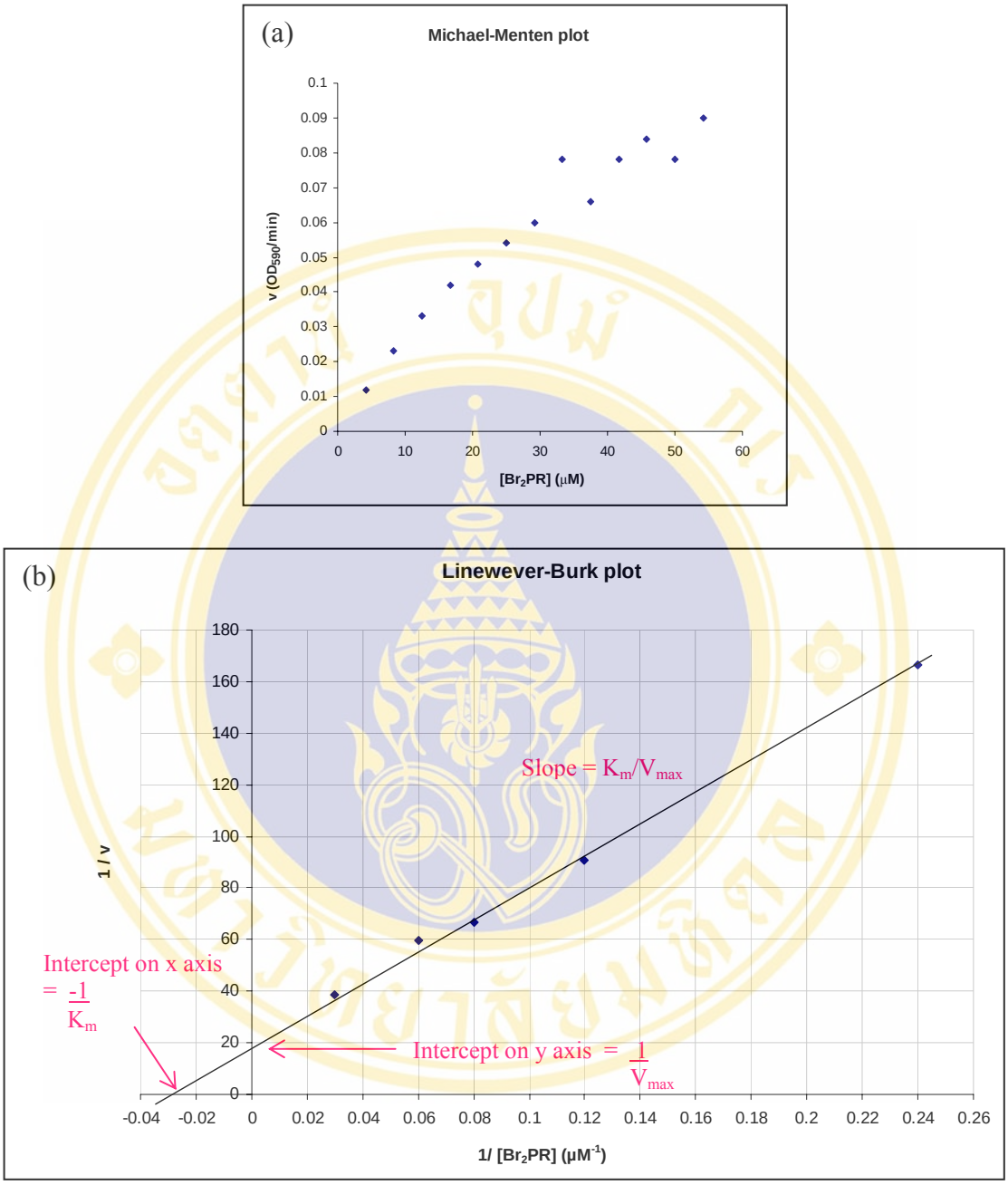

Figure 4-6 (a) Activity of bromoperoxidase at various concentrations of Br_2PR (ranged from 4.17 to 54.17 μM) in the 3 ml reaction mixture containing 20 mM KBr, 0.2 mM H_2O_2 in 0.1 M acetate buffer pH 6.5, and 0.5 ml crude enzyme (0.1 mg protein, 0.1 U/mg protein) at 25°C. Activity (initial rate of the reaction) of bromoperoxidase was plotted against Br_2PR concentrations (Michaelis-Menten plot). (b) The reciprocal plot of the initial rate of bromoperoxidase and Br_2PR concentrations (Lineweaver-Burk plot).

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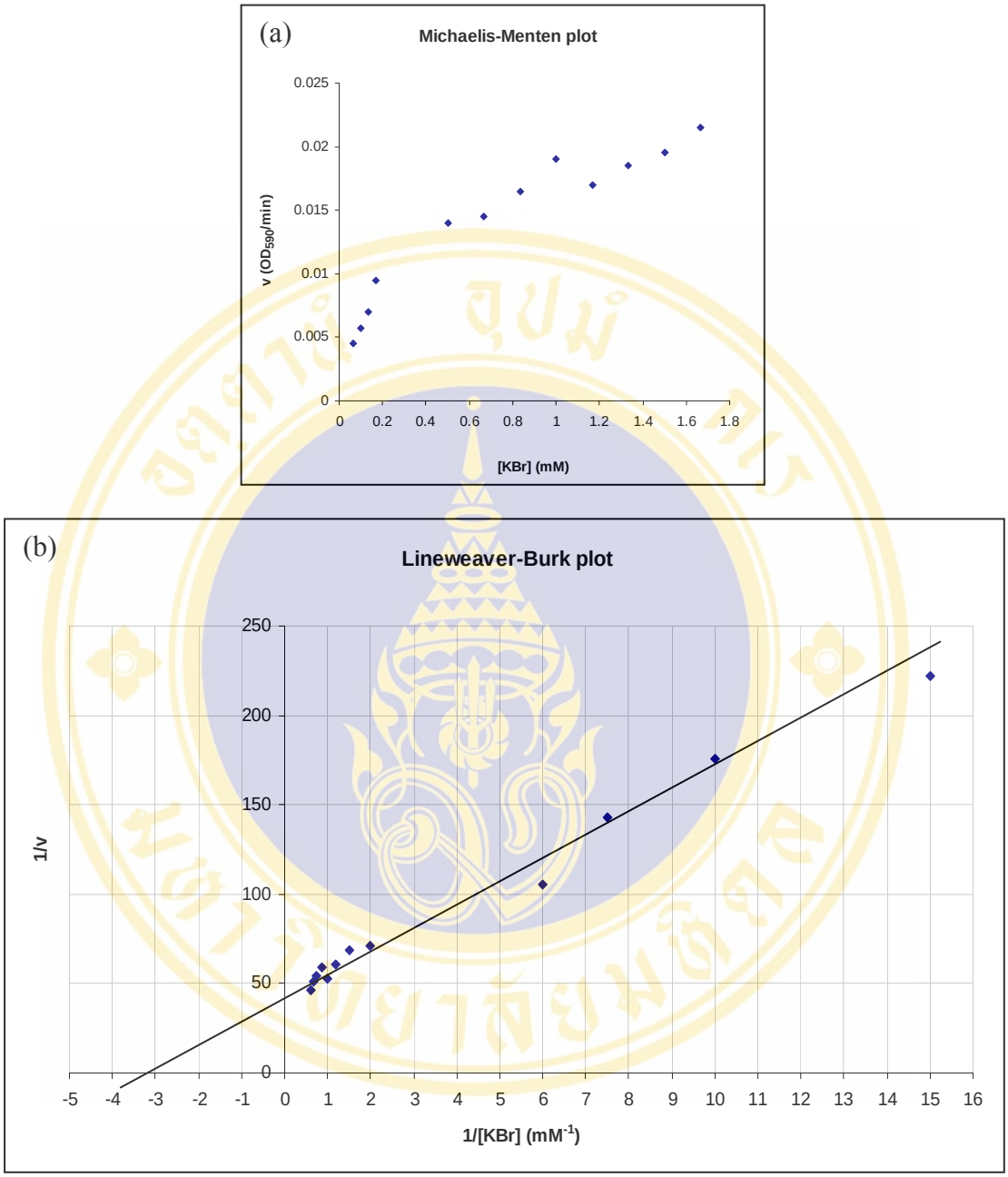

Figure 4-7 (a) Activity of bromoperoxidase at various concentrations of KBr (ranged from 0.07 to 1.67 mM) in the reaction mixture containing 33.33 µM Br_2PR, 0.2 mM H_2O_2 in 0.1 M acetate buffer pH 6.5, and 0.5 ml crude enzyme (0.1 mg protein, 0.1 U/mg protein) of at 25°C. Activity (initial rate of the reaction) of bromoperoxidase was plotted against KBr concentrations (Michaelis-Menten plot). (b) The reciprocal plot of the initial rate of bromoperoxidase and KBr concentrations (Lineweaver-Burk plot).

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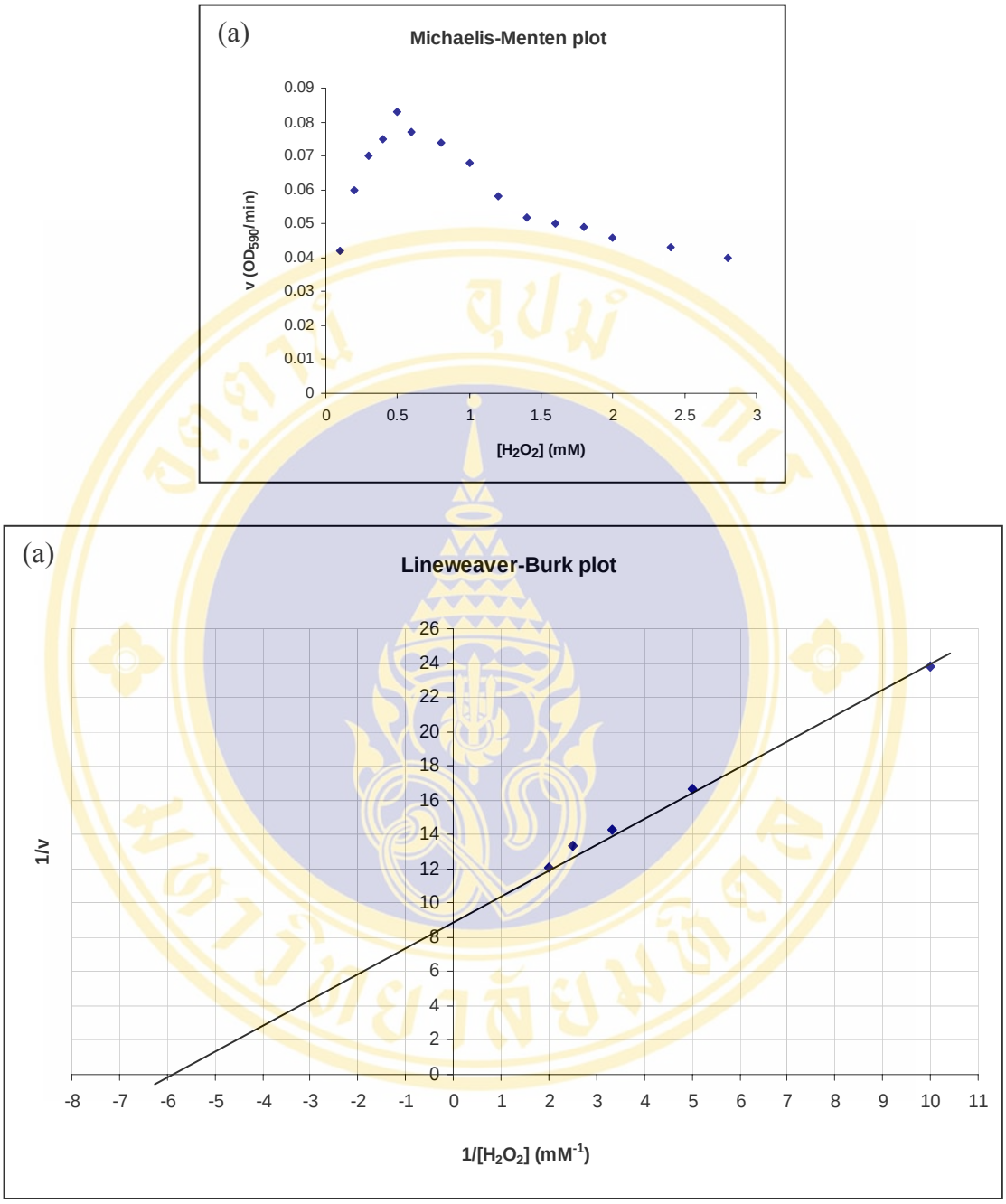

Figure 4-8 (a) Activity of bromoperoxidase at various concentrations of H_2O_2 (ranged from 0.1 to 2.8 mM) in the reaction mixture containing 20 mM KBr, 33.33 µM Br_2PR in 0.1 M acetate buffer pH 6.5, and 0.5 ml crude enzyme (0.1 mg protein, 0.1 U/mg protein) at 25°C. Activity (initial rate of the reaction) of bromoperoxidase was plotted against H_2O_2 concentrations (Michaelis-Menten plot). (b) The reciprocal plot of the initial rate of bromoperoxidase and H_2O_2concentrations (Lineweaver-Burk plot).

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4.1.9 Storage Stability of Red Seaweed Bromoperoxidase

As shown in Figure 4-9, the activity of red seaweed bromoperoxidase (crude extract) decreased with storage time. The enzyme kept at 4°C for 2 days lost about 50% of the activity. Keeping the enzyme at 4°C for 17 days resulted in 67% activity lost.

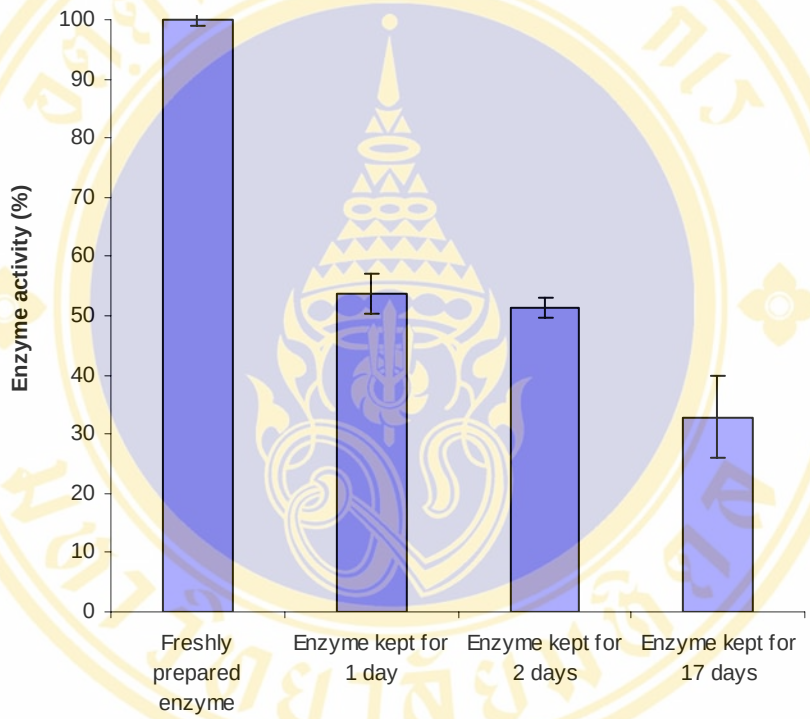

Figure 4-9 Activity of red seaweed bromoperoxidase (crude extract) kept at 4°C for 1-17 days. Activity of crude enzyme was determined as described in section 3.3.1.7.6. Error bars in the graph show standard deviation of a triplicate results.

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4.1.10 Temperature Stability of Red Seaweed Bromoperoxidase

As shown in Figure 4-10, red seaweed bromoperoxidase (crude extract) kept at 4°C showed the highest activity (initial rate = 0.26 OD_{590}/min). After incubation at temperature 34°C and 62°C for 5 min, bromoperoxidase activity decreased to 70% and 8% of the total activity, respectively. Bromoperoxidase activity was completely lost after keeping at 94°C for 5 min.

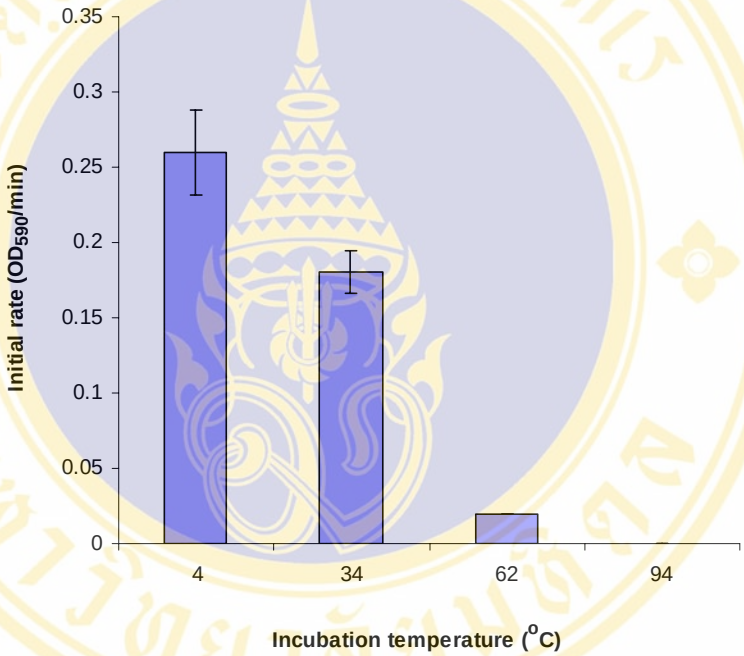

Figure 4-10 Effects of temperature on red seaweed bromoperoxidase activity. The crude enzymes were incubated at 4°C, 34°C, 62°C and 94°C for 5 min in a water bath, before measuring the remaining activity as described in section 3.3.1.7.7.

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4.1.11 Effects of V_2O_5 on Red Seaweed Bromoperoxidase Activity

The addition of V_2O_5 into the enzyme assay showed different effects on the freshly prepared enzyme and the enzyme kept at 4°C for 1 day.

For the freshly prepared red seaweed bromoperoxidase, its activity increased 29% upon addition of 41.67 µM V_2O_5 into the assay mixture. Addition of higher concentration of V_2O_5 resulted in a decrease in the enzyme activity, i.e., 78%, 63% and 40% at 125, 167 and 333 µM V_2O_5, respectively (Figure 4-11).

When the enzyme was kept for 1 day, 46% of the activity was lost. However addition of V_2O_5 at concentration of 41.67 to 333 µM restored the enzyme activity from 14 to 41% (Figure 4-11).

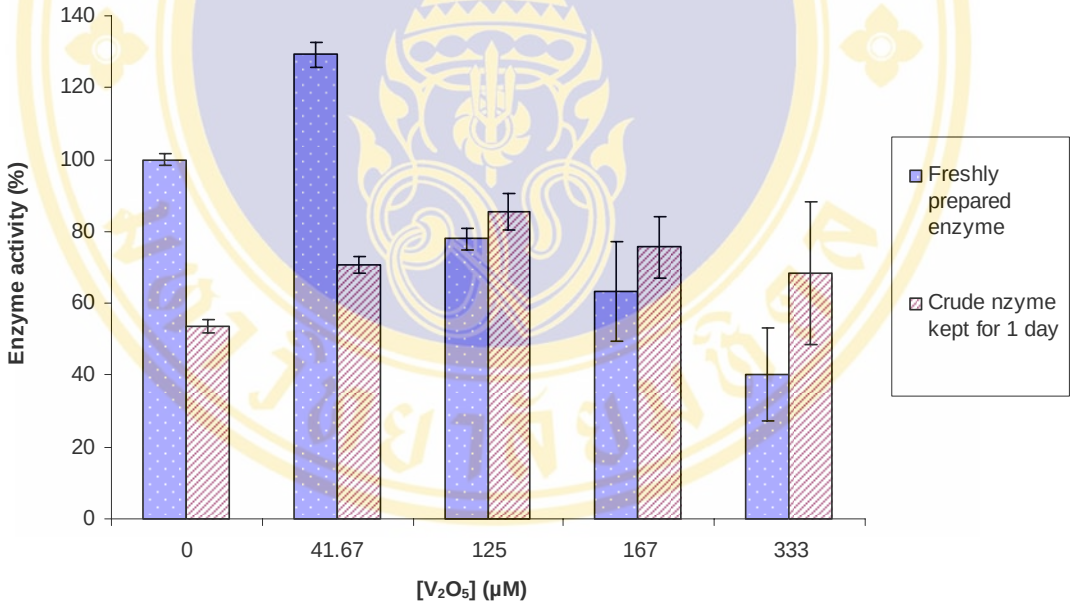

Figure 4-11 Effects of V_2O_5 on the activity of crude red seaweed bromoperoxidase. V_2O_5 at concentrations from 41.67 µM to 333 µM were added to the assay mixture (total volume 3 ml) containing 0.5 ml crude enzyme (0.1 mg protein, 0.1 U/mg protein), 20 mM KBr, 33.33 µM Br_2PR and 1 mM H_2O_2 in 0.1 M sodium acetate buffer pH 6.5. The initial rate of bromoperoxidase-catalyzed reaction was measured as described in section 3.3.1.7.8. Error bars in the graph show standard deviation of a triplicate results.

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4.1.12 Effects of NaN_3 on Red Seaweed Bromoperoxidase Activity

As shown in Figure 4-12, activity of crude red seaweed bromoperoxidase decreased with increase concentration of NaN_3. About 50% of the enzyme activity was inhibited at 0.064 mM NaN_3. Only 5% of the enzyme activity was left at 1.28 μM NaN_3.

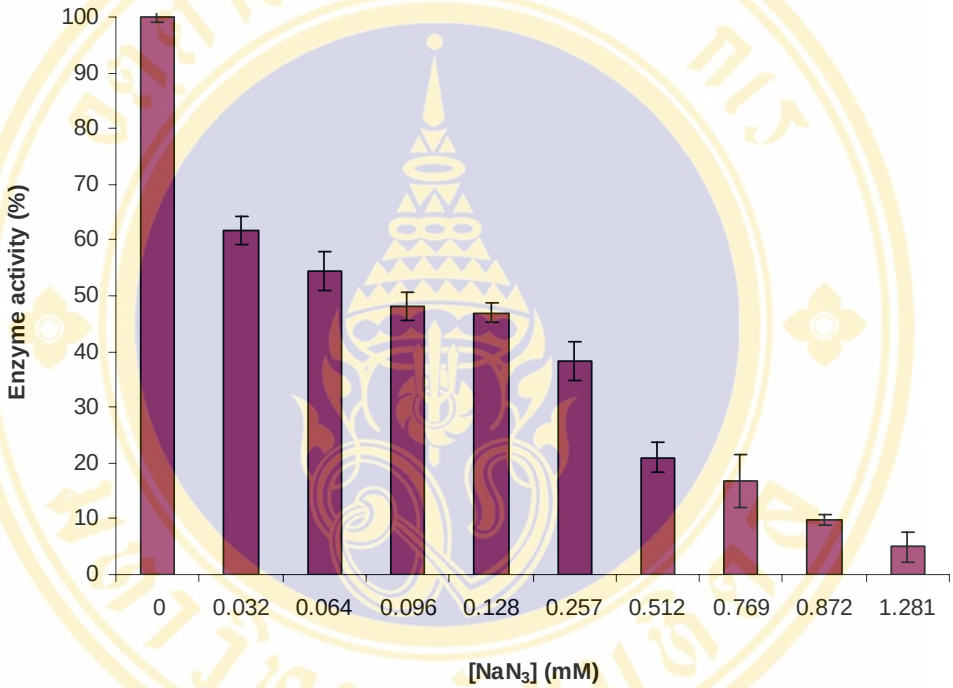

Figure 4-12 Effects of NaN_3 concentrations on the activity of crude red seaweed bromoperoxidase. NaN_3 at concentrations from 0.032 mM to 1.281 mM were added to the assay mixture (total volume 3 ml) containing 0.3 ml crude enzyme (0.06 mg protein, 0.1 U/mg protein), 20 mM KBr, 33.33 μM Br_2PR and 1 mM H_2O_2 in 0.1 M sodium acetate buffer pH 6.5. The bromoperoxidase activity was measured as described in section 3.3.1.7.9. Error bars in the graph show standard deviation of a triplicate results. Error bars in the graph show standard deviation of a triplicate results.

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4.1.13 Studies on Mechanism of Inhibition of NaN_3 at Fixed Concentration of KBr

The Lineweaver-Burk plot of 1/v vs. 1/[KBr] at all concentrations of NaN_3 were linear with different V_{max} (different intersect on the y axis). All the lines intersected on the x axis giving rise to a single K_m (Figure 4-13a), indicating that NaN_3 acted as non-competitive inhibitor with KBr. The pattern of Dixon plot of 1/v vs. [NaN_3] at fixed concentrations of enzyme and KBr (Figure 4-13b) confirmed that NaN_3 was a non-competitive inhibitor with KBr. The inhibitor constant (K_i, inhibitor concentration which halves the value of V_{max}) was found to be 0.07 mM.

4.1.14 Studies on Mechanism of Inhibition of NaN_3 at Fixed Concentration of Br_2PR

The Lineweaver-Burk plot of 1/v vs. 1/[Br_2PR] at all concentrations of NaN_3 were linear with different V_{max} (different intersect on y axis). All the lines intersected on x axis giving rise to a single K_m (Figure 4-14a), indicating that NaN_3 acted as non-competitive inhibitor with Br_2PR. The pattern of Dixon plot of 1/v vs. [NaN_3] at fixed concentrations of enzyme and Br_2PR (Figure 4-14b) confirmed that NaN_3 was a non-competitive inhibitor with Br_2PR. The inhibitor constant (K_i, inhibitor concentration which halves the value of V_{max}) was found to be 0.17 mM.

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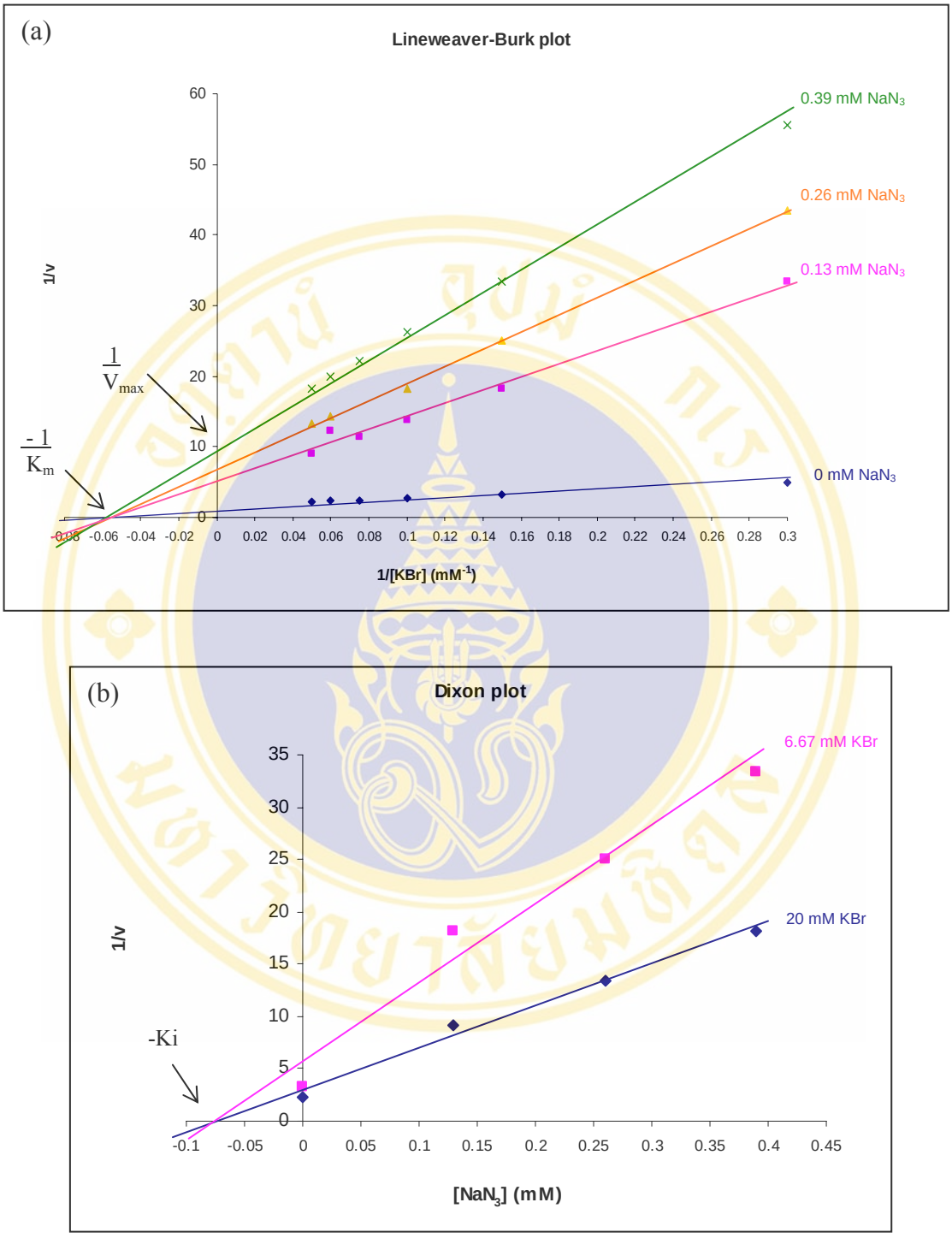

Figure 4-13 (a) the Lineweaver-Burk plot of 1/v against [NaN_3] at fixed enzyme and [KBr]. The assay was performed at 25°C by using 0.5 ml crude bromoperoxidase in the reaction mixture (total volume 3 ml) containing 33.33 µM Br_2PR and 1 mM H_2O_2 in 0.1 M sodium acetate buffer pH 6.5, and (b) Dixon plot showing the effect of various NaN_3 concentrations at fixed enzyme.

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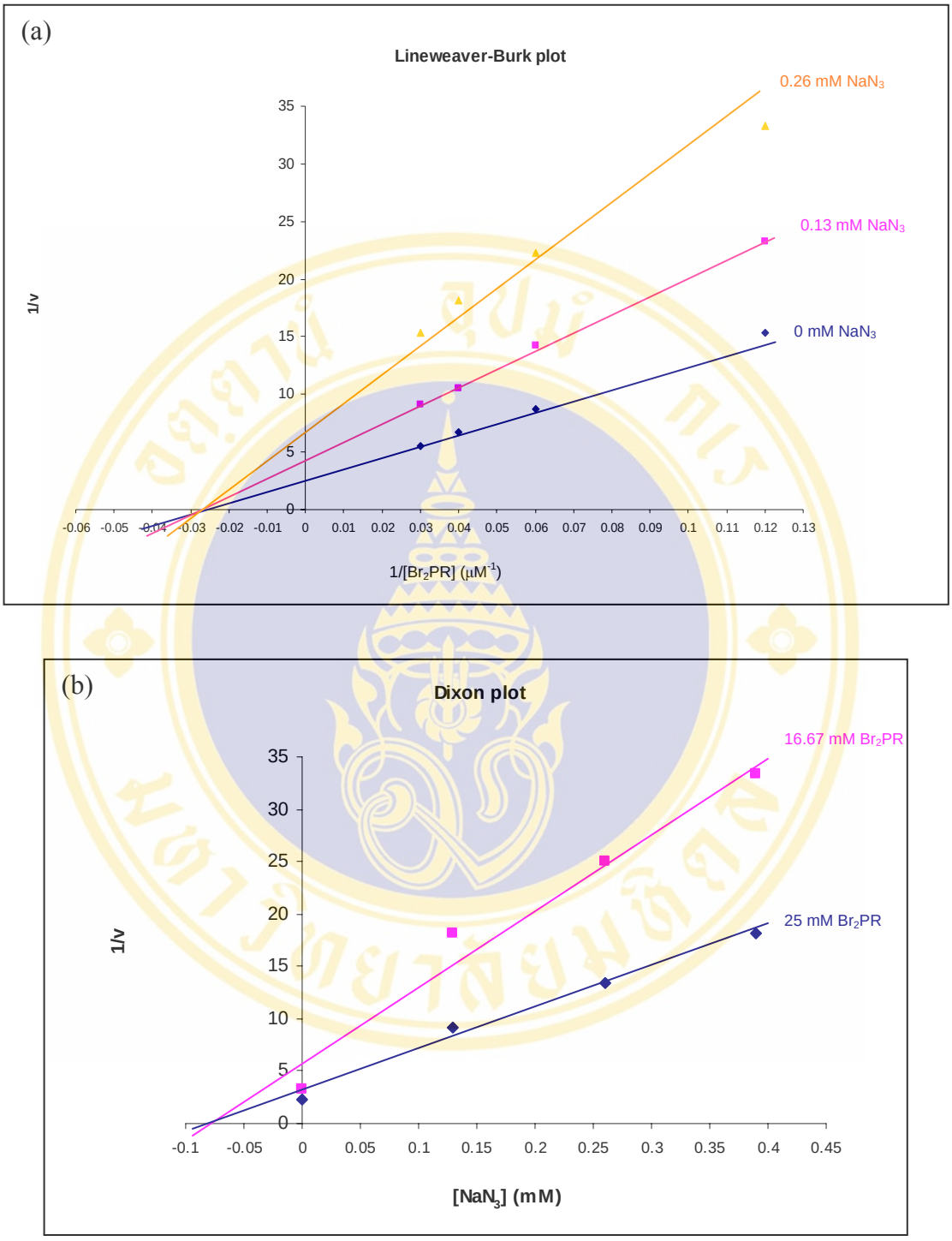

Figure 4-14 (a) the Lineweaver-Burk plot of 1/v against [NaN_3] at fixed enzyme and [Br_2PR]. The assay was performed at 25°C by using 0.5 ml crude bromoperoxidase in the reaction mixture (total volume 3 ml) containing 20 mM KBr and 1 mM H_2O_2 in 0.1 M sodium acetate buffer pH 6.5, and (b) Dixon plot showing the effect of various NaN_3 concentrations at fixed enzyme.

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4.1.15 Agar Gel Testing for Red Seaweed Bromoperoxidase

When the cut red seaweed *G. tangi* was placed into the agar slide containing Br_2PR, the pink color in agar around the seaweed changed to blue color within a few minute. The resulted indicated the presence of bromoperoxise activity of the red seaweed as observed from a dark blue fringe developed in the gel around each frond against a pink background for Br_2PR (Figures 4-15a and 4-15b). Prolonged immersion of the red seaweeds in the gel turned the whole slide blue (Figure 4-15c). Red seaweed boiled for 30 min did not cause color change in the agar gel around it (Figure 4-15d).

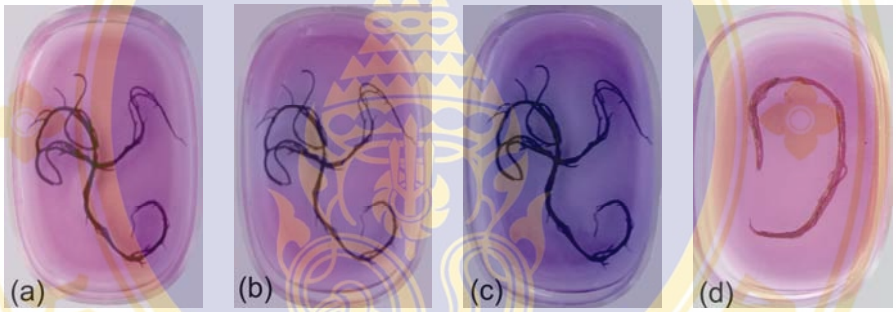

Figure 4-15 *Gracilaria tangi* in agar-kit slide (a) at time 0, no blue color development, (b) at time 10 min, deep blue color expanding fringes due to product(s) of bromoperoxidase activity on Br_2PR, (c) at time 60 min, total coverage of blue colored product(s), and (d) boiled seaweed in agar-kit slide for 30 min generating no blue color around it.

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4.2 Educational Part

The learning cycle based on seaweed bromoperoxidase was implemented in the undergraduate science class. The effectiveness of this learning model on student's understandings of enzyme kinetics as well as on their perceptions and preferences were investigated. The results of this study were reported in five parts. The first part describes the effects of the learning cycle based on seaweed bromoperoxidase experiment on students' understanding of the enzyme kinetics concepts. The second part describes the effects of the learning cycle on student perceptions and their preferences of the instructional learning activities. The third part describes the teachers' perspective toward the learning cycle approach. These include advantages, disadvantages, and some difficulties in using the learning cycle approach from their perspectives. The fourth part describes what occurred on in the learning cycle class focusing on interaction between student-student and student-teacher. The fifth part summarizes the overall picture of these findings.

4.2.1 Effects of the Learning Cycle based on the Developed Bromoperoxidase Experiment on Students' Understanding of Enzyme Kinetics

The effects of the learning cycle based on bromoperoxidase experiment on students' understanding of enzyme kinetics were evaluated using various measures: pre- and post-test analysis of Enzyme Conceptual Achievement Test (ECAT), Enzyme Laboratory Achievement Test (ELAT), as well as concept mapping. In addition, student documents such as laboratory work sheets, laboratory reports, their post-laboratory assignments, and their poster presentations were considered for triangulation with the first three measures. Here, the result of each measure was described separately.

4.2.1.1 Findings from ECAT

Enzyme Conceptual Achievement Test (ECAT) was used to measure the students' conceptual understanding of enzyme-catalyzed reaction at various times; at the beginning of teaching and learning of this topic (pre-test), at the end of exploration phase (laboratory class) (post-test 1), at the end of concept introduction phase or

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lecture class (post-test 2), and at two months after the learning cycle class (retention test). The alpha reliability for the overall items of the test was 0.92. The internal reliabilities for each key concept were shown in Table 4-1.

Table 4-1 The internal reliabilities of ECAT regarding three key concepts of enzyme kinetics

Key concepts of enzyme kinetics	Test items	Alpha reliability
1. Nature of Enzyme	1, 2, 4-7	0.83
2. How Enzymes Work	3, 8, 9, 10	0.67
3. Influence of Physical and Chemical Environments on Enzymes' Function	11-18	0.84
Overall	1-18	0.92

4.2.1.1.1 Students' understanding of overall concepts of enzyme kinetics

The students' average score before and after they had learned the concepts of enzyme kinetics in the learning cycle class was shown in Figure 4-16. Their average scores gradually increased after they had performed enzyme kinetics experiments (exploration phase) and had participated in knowledge construction (concept introduction phase). That was, after they had performed the enzyme kinetics experiments, the post-test 1's average score was 28.67 ± 7.44; which was 3.42 points higher than that of the pre-test's average score (25.25 ± 6.46). This difference was statistically significant. After they had participated in knowledge construction and were taught the concept in the lecture class, the post-test 2's average score was 36.96 ± 6.71; which was 8.29 points higher than post-test 1's average score. This difference also, was statistically significant. Two months after this class, the students' retention test average score was 34.23 ± 6.99 which decreased 2.73 points from post-test 2's average score. However, statistically, the scores between these two tests (post-test 2 and retention test) were not significantly different. These results suggested that students' understanding in enzyme kinetics concepts was improved during the learning cycle class.

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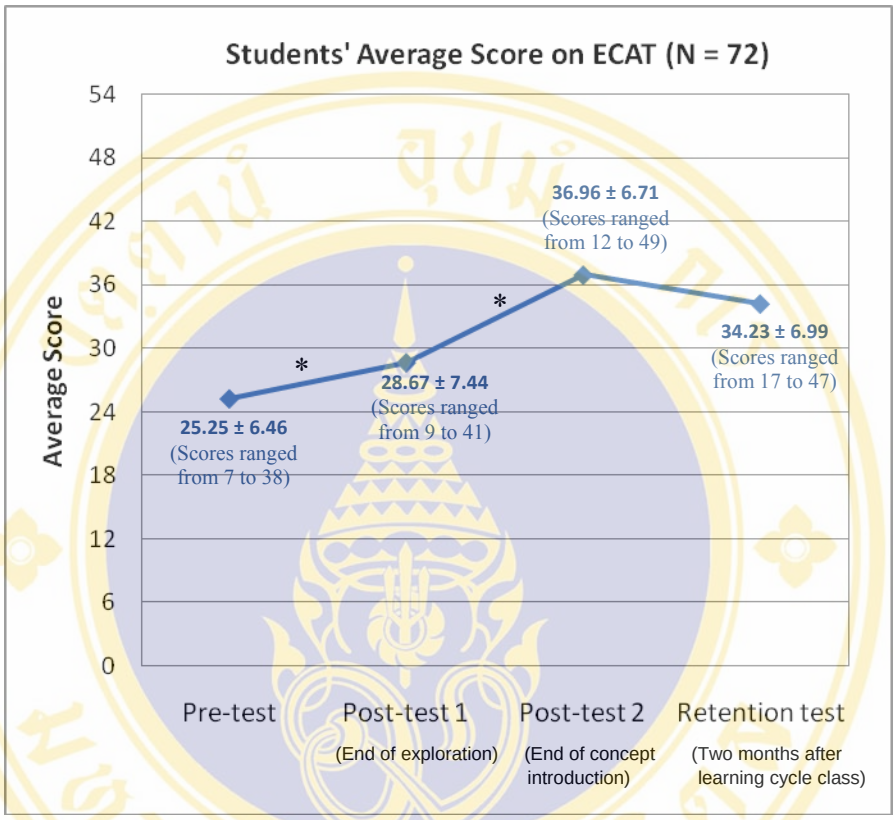
Regarding the three key concepts of enzyme kinetics, students' understandings in each key concept were described next.



* Significant difference ($\rho < 0.05$)

Figure 4-16 Students' average score on ECAT before and after learning of enzyme kinetics in the learning cycle class (Total score for ECAT was 54)

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4.2.1.1.2 Students' understanding in the Nature of Enzyme

The results as shown in Figure 4-17 suggested the teaching and learning of enzyme kinetics in this class increased students' understanding in the nature of enzymes. The proportion of students' understanding this concept increased significantly and at the same time, the proportion of students' non-understanding decreased significantly. Two months after this class, the proportion of students' understanding did not change.

Sixty-two percents of the students (total 72) had better understanding in the nature of enzymes after they had learned in this class, but 33% of the students still had partial understanding in this concept. They did not respond badly on items 1, 2, 4, and 5 but had more problems on items 6 and 7 (see data in Appendix J). Most students had serious difficulties in understanding the binding between enzyme and substrate (item 6). They also had difficulties in understanding the structure of the enzymes and their active site (item 7). Few students had problems with the role of enzymes in living organisms (item 2).

Students' responses to item 6 suggest that most students had difficulty in understanding the binding between enzyme and substrate. There were 87.4% students who thought that binding between an enzyme and substrate is similar to the situation where a key fits only a particular lock. They did not think that the chemical bonds formed between the enzyme and substrate are also important in binding between enzyme and substrate (see data in Appendix J and ECAT items in Appendix A).

The results from item 7 suggested that although students knew the shape of an enzyme and its active site depends on the amino acid functional group of the enzyme, they did not give the appropriate reason. 27.1% students thought that is because each enzyme is involved in different reactions, so they have different shapes and active sites. 14.6% students thought that the amino acid functional group in an enzyme is not involved in the enzyme structure since each enzyme has a specific shape (see data in Appendix J and ECAT items in Appendix A).

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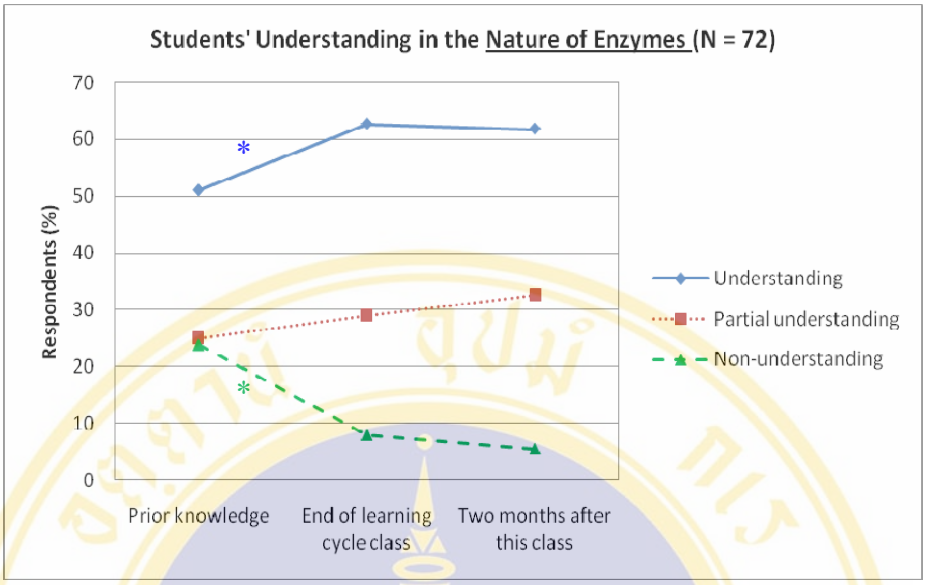

* Significant difference ($\rho < 0.05$)

Figure 4-17 Students' understanding in the nature of enzymes

4.2.1.1.3 Students' understanding in How Enzyme Work

The results shown in Figure 4-18 suggested that teaching and learning of enzyme kinetics in this class increased students' understanding in the concept of how enzymes work. The students' understanding of the concept has increased in a statistically significant level, while the decrease in non-understanding was also at a statistically significant level. Two months after this class, the proportion of students' understanding did not change, but the proportion of partial understanding was statistically significantly higher than that of the end of the class. This was because some students had, by the end of the class, moved from non-understanding to partial understanding.

Although this class promoted student understanding of the concept of how enzymes work, this concept was still difficult for many students to understand. There were 35.26% students had partial understanding, while 18.01% students did not understand this concept. Responses on ECAT suggested that many students had problems with items 3, 8, and 10. Few students had problems with item 9 (see data in Appendix J).

The results from item 3 suggested that many students had difficulties in understanding the process that enzymes act on substrate molecules. There were 54.2%

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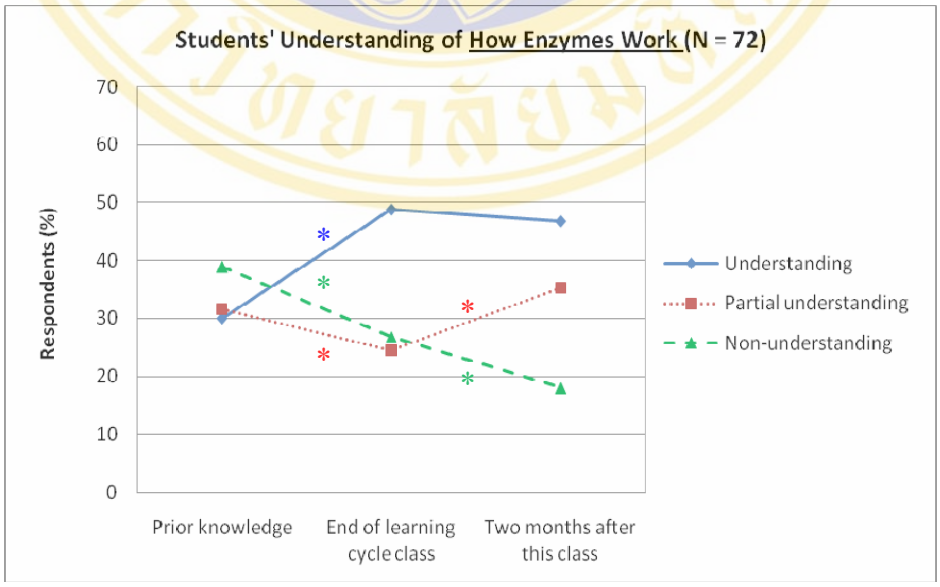
students thought that enzymes only speed up the reaction and do not affect the structure of substrate at all. There were 10.4% students knew that enzymes break down big molecules to yield small molecules but they did not identify that this is because the chemical bonds between small subunits of substrate molecules were broken by enzymes (see data in Appendix 10 and ECAT items in Appendix A).

For item 8, the results suggested that many students did not understand how enzymes make a reaction occur faster. There were 39.6% students thought that enzymes increase the rate of molecular movement during reactions so that reaction occurs faster. There were 18.7% students thought that enzymes change ΔG for a reaction, making it more negative (see data in Appendix J and ECAT items in Appendix A).

For item 10, the results suggested that many students had difficulty in understanding that the rate of enzyme reaction can change over time since the enzyme may denature with time or the substrate will gradually decrease, resulting in a decrease in the rate of reaction. Instead, 27.1% students thought that the rate of an enzyme-catalyzed reaction is constant over time, because enzymes are never used up (see data in Appendix J and ECAT items in Appendix A).



* Significant difference ($\rho < 0.05$)

Figure 4-18 Students' understanding of how enzymes work

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4.2.1.1.4 Students' understanding of the Influence of Physical and Chemical Environments on Enzyme Functions

The results shown in Figure 4-19 suggested that after the students had learned the enzyme kinetics topic in this class, they had better understanding of the influence of physical and chemical environments on enzyme functions. The students' understanding of the concept has increased in a statistically significant level, while the decrease in non-understanding was also at a statistically significant level. After this class, some students forgot what they had learned so that they responded incorrectly resulting in an increase in the proportion of non-understanding. This different was statistically significant.

Although this learning cycle increased the understanding of more students in the influence of physical and chemical environments on enzymes' function, some students did not have sound understanding since they forgot what they had learned. The responses at the end of this evaluation indicated that 32% students had only partial understanding. These students did not have more problems in understanding the influences of reactants concentration on the rate of enzyme-catalyzed reaction. Rather they had difficulties in understanding the influences of temperature, pH, and inhibitors on enzymes' function (see data in Appendix J and ECAT items in Appendix A).

The responses on ECAT, items 11, 12, and 13, also suggested that many students had difficulties in understanding the effect of temperature on enzymes' function (see data in Appendix J and the ECAT items in Appendix A). Most of them did not know that each enzyme works well at its optimal temperature. Some thought that enzymes work well at 37^{o}C because this is body temperature. Some others thought that enzymes do not work or work slowly at 0^{o}C because enzyme denatured at this temperature. In addition, some students seemed to be transferring knowledge from chemical kinetics to enzyme kinetics; they thought that the rate of enzyme-catalyzed reaction always increases with temperature because higher temperatures make molecules move faster.

For item 14, the results suggested that although students knew acidic or basic environments (pH) influence enzyme activities, they did not give the appropriate

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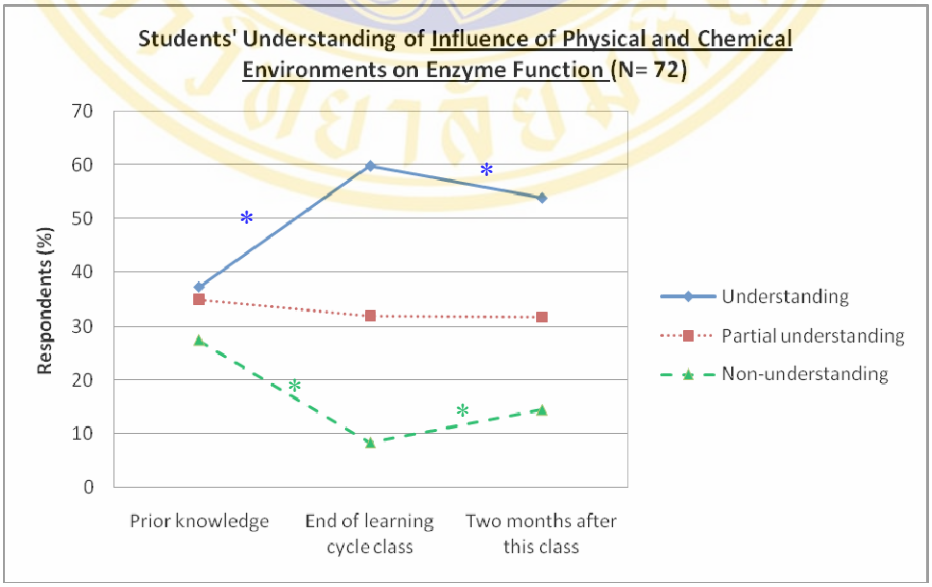
reason that changing the pH affects the electric charge of the substrate and of the amino acid functional group of the enzyme active site, resulting in the enzyme cannot bind to the substrate. Some students (8.3%) thought that enzymes are destroyed under conditions of high acidity or basicity while the others (8.3%) thought that enzymes are denatured at only low pH not at high pH. There were 37.5% students thought enzymes work well at pH 7.0, giving the reason that the pH in the body is neutral and most enzymes operate in the human body (see data in Appendix J and ECAT items in Appendix A).

For items 17 and 18, the results suggested that although students knew that inhibitors reduce enzyme activity, many students were still confused. There were 47.9% students who thought that inhibitors reduce enzyme activity since an inhibitor binds with the substrate (rather than binding with the enzyme) meaning that enzymes cannot bind with the substrate (item 17) (see data in Appendix J and ECAT items in Appendix A). The results from item 18 also suggested that some students did not know exactly what inhibitors are, since they did not know that a substance with similar structure to the substrates and can bind with the active site of enzyme, acts as an inhibitor (see data in Appendix J and ECAT items in Appendix A).



* Significant difference ($\rho < 0.05$)

Figure 4-19 Students' understanding of the influence of physical and chemical environments on enzyme functions

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In summary, the learning cycle based on bromoperoxidase experiment seemed to be effective in promoting student understanding in the concepts of enzyme kinetics. More students had better understanding of these concepts after they had learned in this class. However, some students still had difficulties in understanding some topics. More data were needed to triangulate with these findings. Next, the findings from ELAT are described.

4.2.1.2 Findings from ELAT

The Enzyme Laboratory Achievement Test (ELAT) was used to measure the students' understanding of the concepts related to the enzyme kinetics laboratory before and after performing the enzyme kinetics experiments. Students' responses to ELAT were scored by two teachers using the same criteria (see Appendix B). The alpha reliability of the test was 0.87.

The responses on ELAT items were classified into six categories according to the objectives of practical work, particular in enzyme kinetics experiments. These were:

(1) identification of enzyme, substrate, and product of an enzyme reaction (items 1.1, 1.2, 1.5, and 1.7);
(2) setting of an hypothesis to test the experiment (item 1.4);
(3) interpretation and conclusions based on the empirical data (item 1.6);
(4) design of an experiment to test the effect of some factors on enzyme reaction (items 1.3, 2.1, 2.2, and 2.3);
(5) calculation of the rates of an enzyme reaction and explanation of the phenomena (items 3.1 and 3.2); and
(6) analysis and discussion of the effects of some factors on the enzyme reactions (items 3.3, 3.4 and 4).

Since the total score of each category was different, the student scores of each category were converted into percentages. This also made the results more easily comparable. The results shown in Figure 4-20 demonstrated that students' post-lab scores in each category of ELAT were higher than their pre-lab scores. These results suggested that after students had performed the enzyme kinetics in this class, they had

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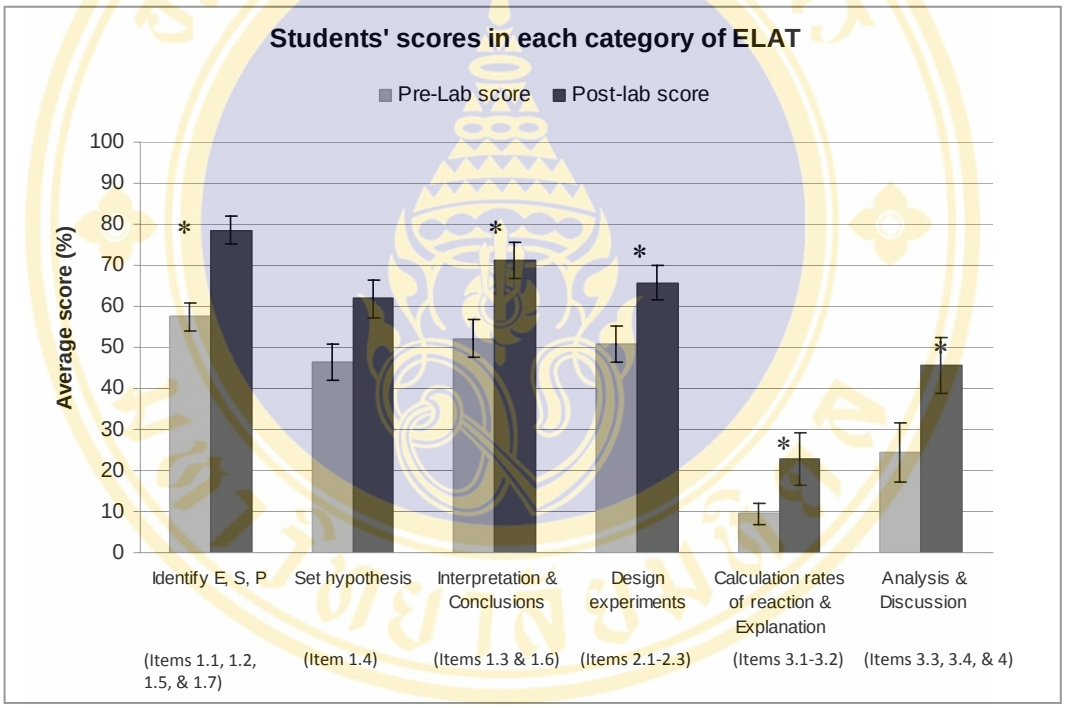
better understanding of the concepts related to enzyme kinetics experiments. They could identify the enzyme, substrate, and product of an enzyme reaction more correctly. They could write an equation of the particular reaction. They could calculate the rates of the enzyme reactions. They could analyze and discuss the effects of some factors (such as pH, temperature, reactant concentration, and inhibitor) on enzyme functions. In addition, their abilities in interpretation and drawing a conclusion, setting an hypothesis, and designing an experiment also increased after they had performed the experiment themselves.



* Significant difference ($\rho < 0.05$)

Figure 4-20 Students' scores in each category of ELAT

Students' post-lab scores on items 1.1, 1.2, 1.5, and 1.7 (see ELAT items in Appendix B) were 78.55%; that was 21.05% higher than their pre-lab score (average 57.5%). This difference was statistically significant (see data in Appendix K). These suggested that after students had experience in the enzyme kinetics laboratory, they had better understanding of the enzyme reaction. They could better identify the enzyme, substrate, and product of the reaction.

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The students' post-lab score for item 1.4 (see ELAT items in Appendix B) averaged 61.94% and the pre-lab score for this item averaged 46.48%. Although this difference was not statistically significant, the post-lab score was 15.16% higher than the pre–lab score (see data in Appendix K). This suggested that after students had the opportunity to perform experiments themselves, they improved their ability to set an hypothesis to test the experiment.

Their post-lab scores for items 1.3 and 1.6 (see ELAT items in Appendix B) averaged 71.27%; that was 19.15% higher than the pre-lab score (average 52.12%). This difference was statistically significant (see data in Appendix K). These scores suggested that after they had performed the experiment themselves with minimal guidance, they increased the students' ability in interpretation of data and drawing a conclusion. They could also write the equation of the enzyme reaction based on the empirical data more correctly than they could before learning in this laboratory class.

Students' post-lab scores for items 2.1 to 2.3 (see ELAT items in Appendix B) averaged 65.77%; that was 15.07% higher than their pre-lab score (average 50.7%). This difference was statistically significant (see data in Appendix K). These suggested that after students had experience in this laboratory class, they improved their ability to design an experiment themselves.

The students' post-lab score for items 3.1 and 3.2 (see ELAT items in Appendix B) averaged 22.97%; that was higher than their pre-lab score (average 9.48%). This difference was statistically significant (see data in Appendix K). However, their post-lab score was very low. Most students calculated an initial rate correctly (item 3.1) but they could not provide appropriate explanation of what went on during the reaction progress (item 3.2). These suggested that these concepts were still difficult for students to understand.

The students' post-lab scores for items 3.3, 3.4, and 4 averaged 45.59%; that was 21.26% higher than their pre-lab score (average 24.33%). This difference was statistically significant (see data in Appendix K). These suggested that students had better understanding of the influence of some factors on enzyme reaction. They could use this knowledge to analyze the effect of an inhibitor on the particular reactions (see ELAT items in Appendix B). However, their post-lab score was lower than 50%.

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Some concepts were difficult for students to understand. They could not give the reason why some factors affected the rate of enzyme reaction (see ELAT items 3.3 and 3.4 in Appendix B). In addition, they had difficulties in understanding the effects of an inhibitor on the whole reaction (see ELAT item 4 in Appendix B)

In summary, the inquiry-based bromoperoxidase laboratory used in the exploration phase of the learning cycle class seems to be effective in improving student ability to set hypothesis, to interpret data and draw a conclusion, and to design an experiment. Students seemed to have better understanding of some concepts related to the enzyme kinetics laboratory but they still had difficulties in explaining the phenomena of what is going on in the enzyme reaction and how some factors change the enzyme functions. However, the data from student laboratory work sheets, posters, laboratory reports, and post-laboratory assignments, needed to be used corroboratively with these findings.

4.2.1.3 Findings from Student's Documents (e.g. Laboratory Work Sheets, Laboratory Reports, Post-Laboratory Assignments, and Poster)

After students had completed their own efforts at performing the experiments, each group of students was asked to prepare a poster to present and discuss in the whole class. After they had been introduced to the key concepts in the lecture class, they were asked to write a group laboratory report and performed a group assignment. The researcher's analyses of their documents suggested that students had abilities in designing their experiments, recording data, plotting graphs, calculating enzyme reaction rates, analyzing data, and drawing conclusions. Some groups also showed ability to use scientific knowledge to support or explain their findings. Students could evaluate their experiment as well. They also could use the knowledge in the new situations. Their documents also showed that students understood the concepts of enzyme kinetics. They understood the concepts of how enzymes work. They knew how the rate of enzyme catalytic reaction is measured. However, some students did not know what area of the graph could be used to calculate the initial rate and they did not seem to understand the meaning of the rate of reaction. Although students knew

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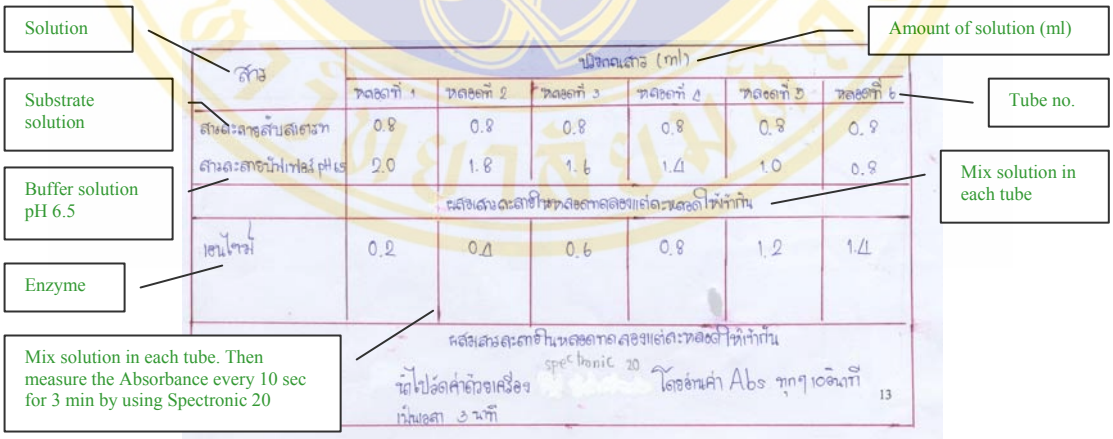
how physical and chemical environments such as pH, the concentration of enzyme or substrate, and the other substances affect the rate of reaction, many students did not provide an explanation of why these factors affect enzyme functions. The details of these results and the examples of the student documents were given next.

4.2.1.3.1 Student's ability in designing an experiment

Analyses of group laboratory worksheets suggested that all groups of students had the ability to design an experiment to investigate the influence of physical and chemical environments such as pH, reactant concentrations, and the other substances on the rate of enzyme reaction.

The figures below show examples of student designs for an experiment to investigate the influence of enzyme concentration (Figure 4-21) and to investigate the effect of pH (Figure 4-22) on enzyme catalytic reaction. Their documents suggested students knew how the progress of enzyme catalytic reaction be measured. They knew what the control of the experiment is. However, some students did not set appropriate hypothesis for their experiment (see example in Figure 4-22).



Students planned their experiment clearly.
They provided logical steps in their experimental design.

Figure 4-21 An example of student group work in designing an experiment to investigate the effects of enzyme concentrations on enzyme catalytic reaction (Source: Group laboratory work sheet)

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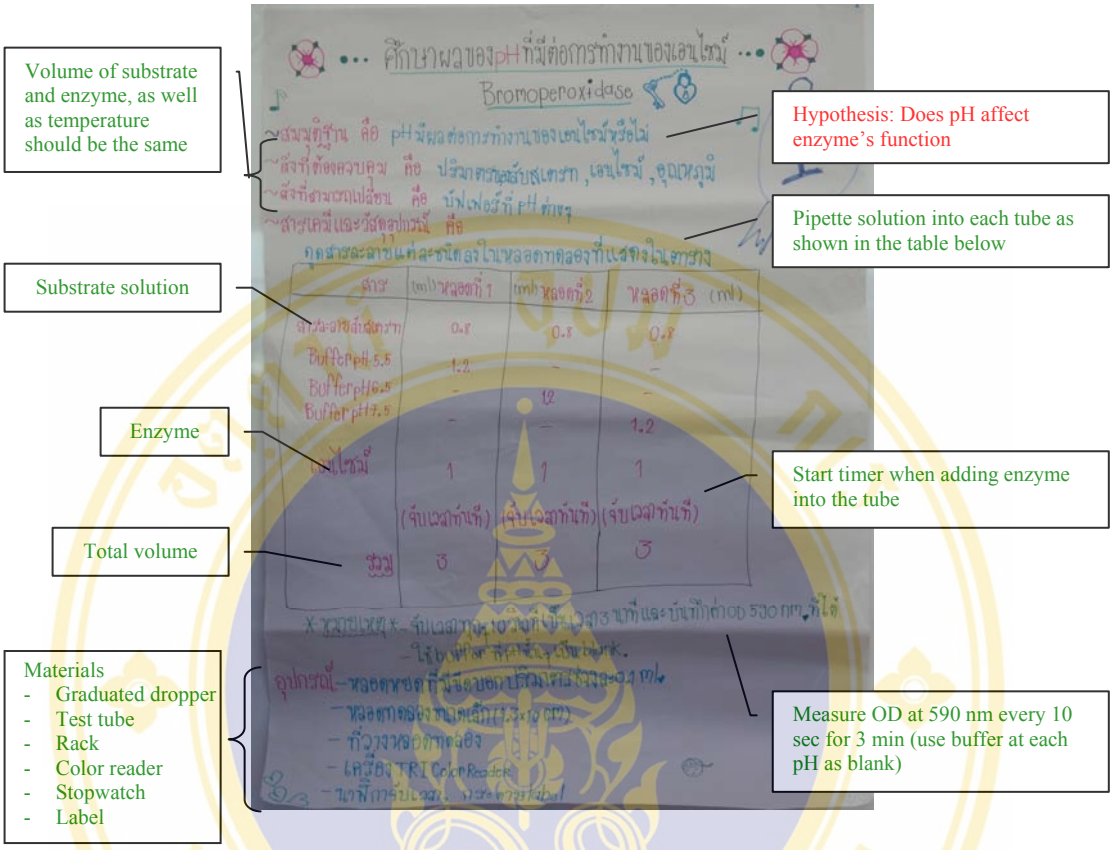

Figure 4-22 An example of student group work in designing an experiment to investigate the effects of pH on enzyme catalytic reaction (Source: Group poster)

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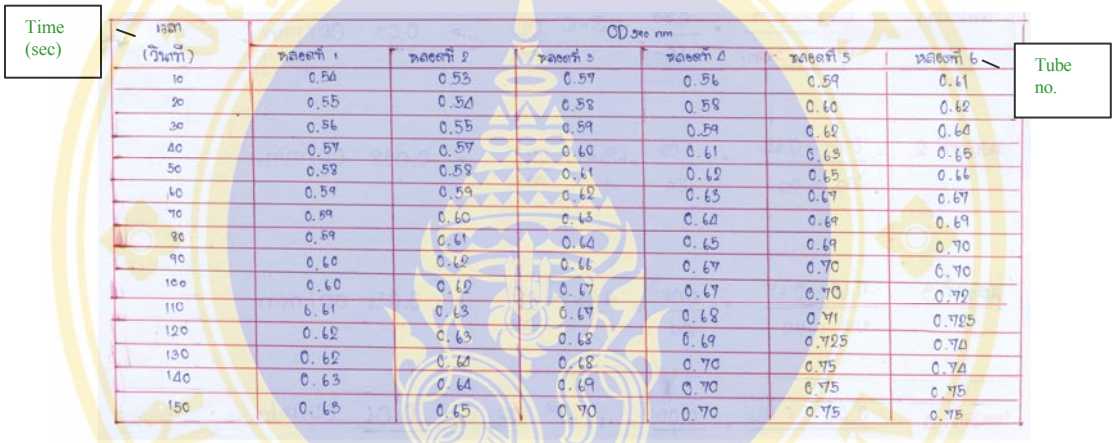
4.2.1.3.2 Student's ability in recording data

The results of group laboratory work sheets suggested that students recorded data systematically. All groups of students recorded data of typically appropriate outcomes that was putting the results of observation or measurement onto paper in a way that others can quickly and easily understand. They presumably followed the procedure that they designed well and had accurate techniques and measurements. They also wrote the unit of the measurements in their data sheets (see an example in Figure 4-23).



Students clearly recorded data systematically. They also wrote the unit of their measurements.

Figure 4-23 An example of student group work in recording data of the experiment (Source: Group laboratory work sheet)

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4.2.1.3.3 Student's ability in calculating rate of enzyme-catalyzed reaction

Once students obtained raw data, they could process by carrying the calculation. The analysis of group laboratory work sheets suggested that all groups were able to carry out the calculation of the rate of the enzyme reaction and to display results as graphs. Most groups could calculate the initial rate correctly. However, some groups did not know which area of the graph could be used to calculate the initial rate since they calculated this value from the wrong area of the graph (see an example in Figure 4-24).

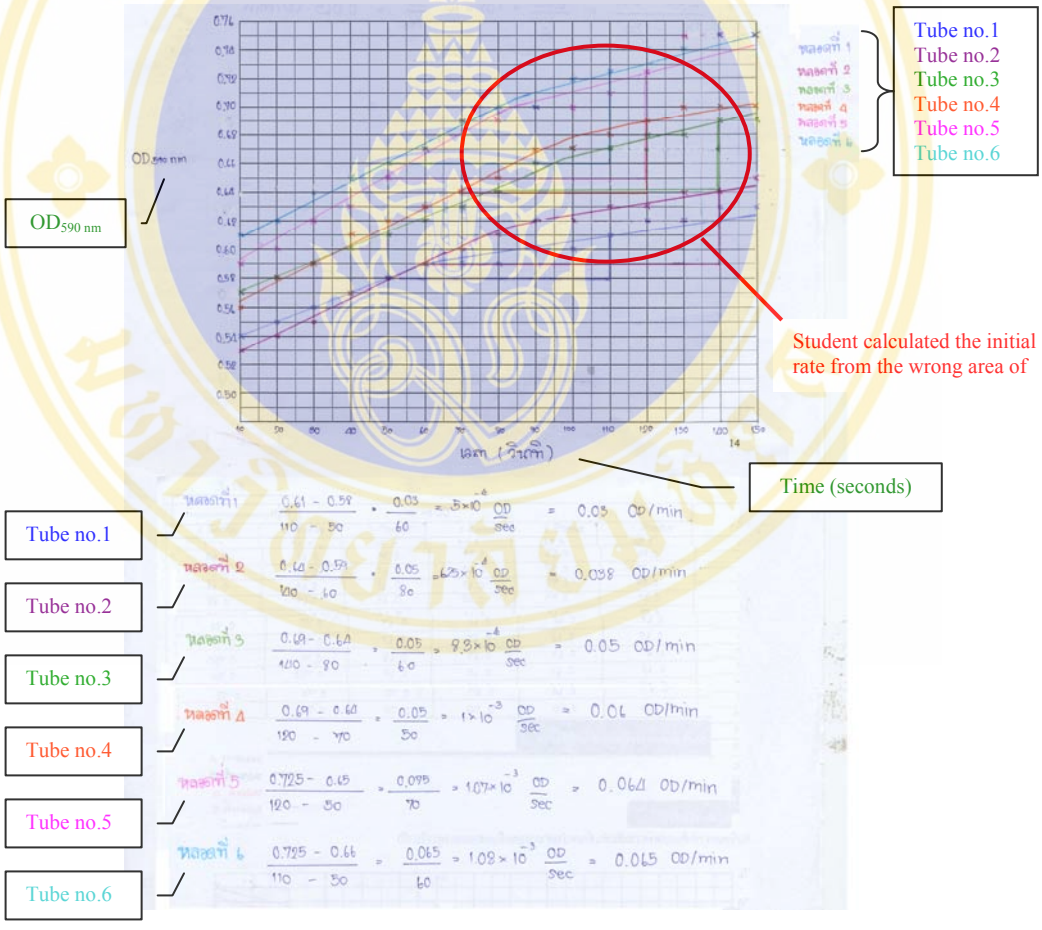

Figure 4-24 An example of student group work in calculating the initial rate (Source:Group laboratory work sheet)

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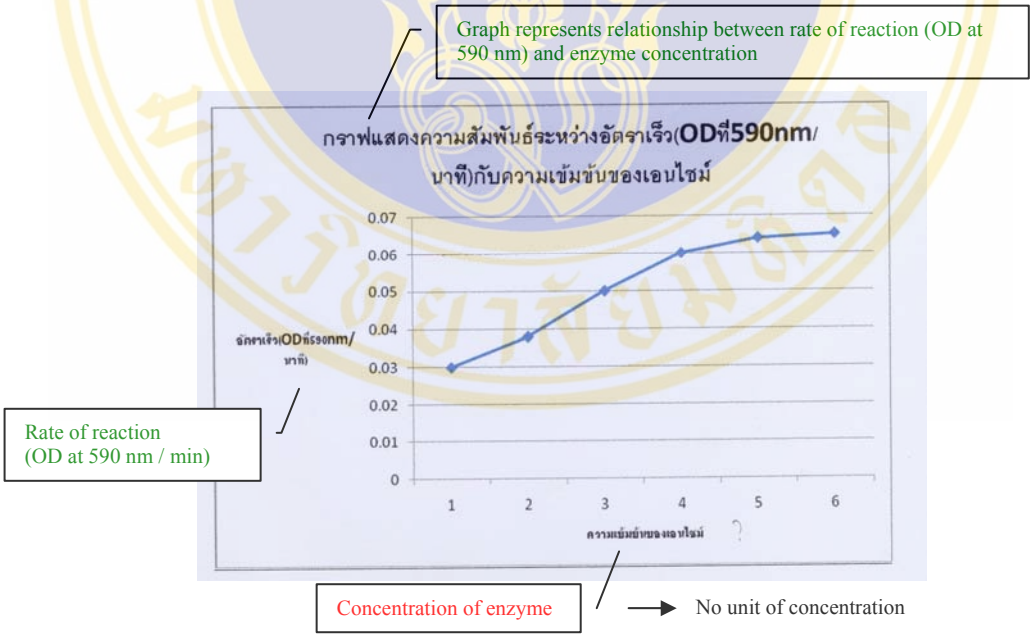
Even though during laboratory work some students did not know where the area of the graphs should be used to calculate the initial rate (as shown an example in Figure 4-24), they responded correctly in item 3.1 of ELAT (this was mentioned in section 4.2.1.2). Students also knew where the area of the graphs has the highest rate or the lowest rate. They seemed to be being understood this after discussion of the results in the whole class at the end of this laboratory class.

4.2.1.3.4 Student's ability in plotting graph

When students were asked to plot a graph to represent the relationships of what they measured, they responded appropriately that was the graph has a title that describes what the graph represents and has axes with labels that include units. However some groups did not write the unit of what they measured such as unit of concentration (see an example in Figure 4-25)



Student's graph has a title that describes what the graph represents and has axes with a label. However, they did not write the unit of enzyme concentration.

Figure 4-25 An example of student work in plotting a graph to represent the relationship between rate of reaction and enzyme concentration (from Excel) (Source: Student laboratory report)

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4.2.1.3.5 Student's ability in interpretation of data

Student documents suggested that students had the ability to analyze and interpret results themselves with minimal guidance. They were able to see what their results mean. They concluded the experiments based on their empirical data. Their documents also suggested that students understood how enzymes worked and the influence of physical and chemical environments on enzymes function.

After students had investigated the enzyme activity in seaweeds by using an agar slide containing substrate of the enzyme, they identified enzyme, substrate, and product of the reaction correctly. They knew that enzyme catalyzes changing of substrate to yield product as they stated that:

> Substrate has pink color, and the product has blue color. The changing of substrate occurs because enzyme (bromoperoxidase) in seaweed catalyzes oxidation reaction of bromophenol red (pink color) to yield bromophenol blue (blue color)
>
> (Source: Student laboratory work sheet)

When they compared the rate of reaction in the presence or absence of enzyme, they knew enzyme speeds up the reaction. They explained that:

> Rates of the reaction in the presence of an enzyme are higher than those of the reaction in the absence of an enzyme. Enzyme speeds up the reaction.
>
> (Source: Student laboratory report)

When they compared the results of the enzyme catalytic reaction at different pH values, they knew enzyme works best at its particular pH. Some groups may compare their results with those of another source as they stated that:

> The pH affects enzyme functions. An enzyme does not work well at too low or too high pH. Each enzyme works best at a different pH. From the result of this experiment, this enzyme works best at pH 6.5 since it has highest rate at this pH.
>
> (Source: Student laboratory report)

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When they compared the enzyme catalytic reaction at different concentrations of substrate or enzyme, they knew the concentrations of these reactants affect the enzyme catalytic reaction. They stated that:

> Substrate concentration affects enzyme reaction. When concentration of substrate increases, this causes products to form rapidly so the rate of reaction increased.
>
> (Source: Student laboratory report)

However, some students did not explain these phenomena appropriately. They explained that:

> Enzyme concentration affects enzyme functions. When concentration of enzyme increases, more enzymes bind with substrate, meaning that product increases.
>
> (Source: Student laboratory report)

When students compared the results of the enzyme catalytic reaction with or without substance that inhibited enzyme's function (students did not know that substance is an inhibitor of the enzyme), they knew that the substance that reduces rate of enzyme catalytic reaction is an inhibitor of that enzyme. They explained correctly that:

> Since the rate of reaction in the presence of sodium azide is lower than those of reaction in the absence of sodium azide, sodium azide inhibits enzyme's function.
>
> (Source: Student laboratory report)

However, their laboratory reports suggested that students did not seem to understand what the absorbance or OD was and did not really understand the rate of reaction. They often used this term to refer to the rate of the reaction, and eventually they represent rate of reaction (OD/min) in the graph (see an example in Figure 4-26).

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Figure 4-26 An example of student group work in concluding the influence of pH on rate of enzyme reaction (Source: Group laboratory report)

4.2.1.3.6 Student's ability in linking scientific knowledge with experience

The analysis of group laboratory reports suggested that some groups show ability to use the knowledge or theory to explain the findings of an experiment. For example, when they compared the rate of reaction in the present or absent enzyme, they concluded that enzyme speeds up the reaction and then used scientific term as "activation energy" to explain this. In their laboratory report, they stated that:

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> Rates of the reaction in the presence of an enzyme are higher than those of the reaction in the absence of an enzyme. Enzyme speeds up the reaction by decreasing activation energy.
>
> (Source: Student laboratory report)

Although some groups did not discuss the influence of physical and chemical environments in their laboratory reports, they discussed it in their poster presentation. Students noted correctly that:

> Sodium azide (inhibitor of the enzyme used in this experiment) reduces the rate of enzyme catalytic reaction because it inhibits binding between enzyme and substrate, meaning that no products are formed.
>
> (Source: Student poster presentation)

4.2.1.3.7 Student's ability in evaluating the results

The analysis of group laboratory reports found that most groups showed the ability to discuss the experimental error of their experiments. For examples:

> The error in measuring the absorption of the products of the reaction may be occurred if the enzyme does not mixed well in the reaction tube.
>
> When enzyme is mixed with the solution in the reaction tube, the absorption of this solution should be measured suddenly otherwise we did not get initial rate.
>
> The initial rate may be an error from measuring of slope from the graph.
>
> (Source: Student laboratory reports)

4.2.1.3.8 Student's ability in using the knowledge

Most groups of students showed the ability to use knowledge in a new situation. When they were asked design an experiment to make more products in a short time with unlimited budget, they considered the factors affected enzyme activity that they had experienced in their experiments. They stated that:

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> To produce more products in a short time, more substrates and enzymes will be used because each enzyme molecule will bind to the substrates and change them to products.
>
> More substrates are used to produce more products. More enzymes are needed to make the reaction occur rapidly. The pH and temperature should be optimized to make enzyme work well.
>
> (Source: Student post-laboratory assignments)

Students also knew that inhibitors could be applied to study metabolic pathways. They stated that:

> The inhibitors are useful. They are used to study the role of the enzyme functions in human body. Inhibitor will stop the reaction that enzyme catalyzes.
>
> (Source: Student post-laboratory assignment)

Students could also use the concepts of the influence of temperature to analyze the situation in their everyday lives. They noted:

> When we have fever and my body temperature is very high, we will be in shock because the reaction in our body may not function properly. This is because the enzymes denature at very high temperature.
>
> (Source: Student post-laboratory assignment)

In addition, students could give the example of the products composed of the enzyme in their local supermarket. They also explained how the enzymes in those products were useful. They stated that:

> The detergent powders contain enzymes. The enzymes are added into these detergents in order to remove dirt such as lipid or proteins from our clothes.
>
> Enzymes from lactobacillus in drinking yogurts help milk digestion in the human body.

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Enzymes in pineapple can be used to digest meat.

(Source: Student post-laboratory assignments)

In summary, an analysis of their documents suggested that students could perform the experiment themselves. Although most groups of students did not set an appropriate hypothesis, they could design the experiment to investigate the influence of physical and chemical environments on enzymes' function, and to interpret data and draw conclusions based on their empirical data. Students understood that:

- enzyme catalyzes change of substrate to yield product;
- enzyme speeds up the reaction by decreasing activation energy;
- the rate of enzyme reaction can be measured by using a simple spectrophotometer;
- each enzyme works best at its particular pH;
- the rate of reaction increases when the concentration of reactants (enzyme or substrate) is increased; and
- an inhibitor reduces the rate of enzyme catalytic reaction.

However, some students seemed to do not understand that:

- the reaction rate is different from the absorption or OD;
- the rate of enzyme reaction determines how fast or slow the formation of product is (not an increase in the amount of product);
- which area of graph could be used to calculate the initial rate of enzyme reaction; and
- why enzyme and substrate concentrations, and pH affect enzyme catalytic reaction.

A further analysis of their documents also suggested that students could apply knowledge they learned into a new situation. They also made connections between the knowledge they learned in the class and their everyday lives.

Next, the findings from students' concept map were described in order to understand the progression of student understanding of the enzyme kinetics.

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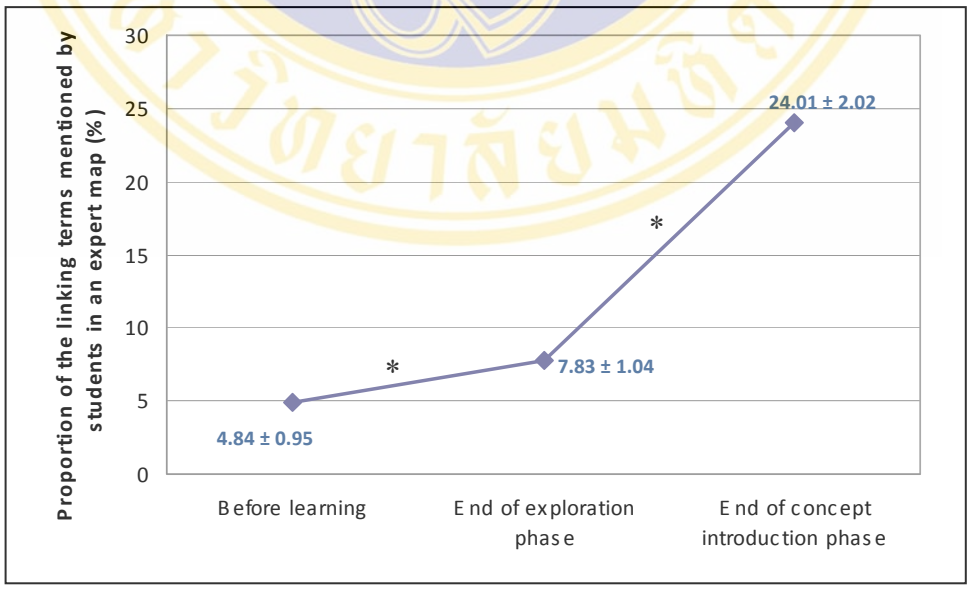
4.2.1.4 Findings from Students' Concept Map

Before students had learned the enzyme kinetics in the learning cycle class, they were asked to draw concept map to represent what they know about the enzymes. At the end of the exploration and concept introduction phases of this class, students were asked to write the concept map again. Then, their concepts maps were compared to an expert map (see an expert map in Appendix H) to assess their understanding of key concepts of enzyme kinetics.

The analyses of students' concept maps suggested that those drawn before learning in this class revealed their prior knowledge as well as some misconceptions of enzyme kinetics. Students knew about the nature of enzymes but very little on the concepts of how enzymes work and the influence of the physical and chemical environment on enzyme functions. However, their knowledge of these concepts increased after they had experience in enzyme kinetics laboratory and learned the concepts in a lecture. Although the teaching and learning strategies used in this class increased students' knowledge of enzyme kinetics, some concepts were still being difficult for them to understand. These findings were shown in details in Figures 4-27 to 4-29.



* Significant difference ($\rho < 0.05$)

Figure 4-27 Proportion of the linking terms mentioned by students in an expert map before and after learning of enzyme kinetics in the learning cycle class (N=72)

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Figure 4-27 demonstrated that the proportion of the linking terms mentioned by students when compared to an expert map before and after the learning cycle. The results suggested the proportion of the linking terms mentioned by students in an expert map increased with phases of the learning cycle. That was, after they had performed the enzyme kinetics experiment (exploration phase), the relevant terms averaged 7.83 ± 1.04 %; that was 2.99 points higher than the relevant terms of those maps drawn before learning in this class (average 4.84 ± 0.95 %). This difference was statistically significant. After they had participated in knowledge construction and were taught the concept in the lecture class (concept introduction phase), the relevant terms averaged 24.01 ± 2.02 %; that was 16.18 points higher than the relevant terms of the maps drawn in the exploration phase. This difference also, was statistically significant. These suggested that student's concepts of enzyme kinetics increased during the learning cycle class (see data in Appendix L).

Figure 4-28 also suggested students' concepts of enzyme kinetics increased during the learning cycle class since the proportion of students whose linking terms were not relevant to an expert map gradually decreased from 50.0% to 3.7% from the beginning to the end of the learning cycle. At the same time, the proportion of students whose linking terms were small relevant to an expert map gradually increased from 48.4% to 72.2% from the beginning to the end. The proportion of students whose linking terms were partially relevant to an expert map also increased from 1.6 % to 22.2% from the beginning to the end. In addition, at the end of the cycle, the linking terms mentioned by one student (1.9% of the class) were substantial relevant to an expert map. These suggested teaching and learning of enzyme kinetics in this class gave students know more concepts of enzyme kinetics (see data in Appendix L).

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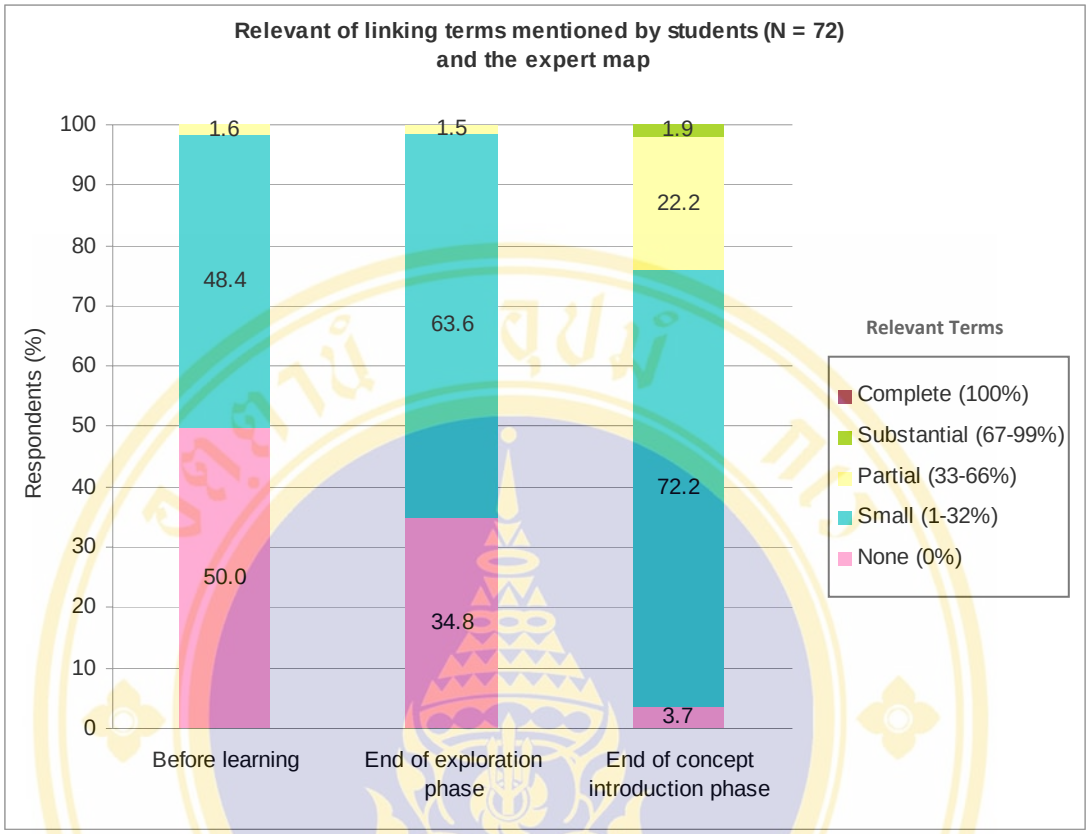

Figure 4-28 Relevant of linking terms in students' concept map compared to an expert map

Students' concept maps also suggested that the concepts of enzyme kinetics were gradually built in each phase of the learning cycle. Figure 4-28 represents an example of the concept maps written by Songkran before and after he had learned in the class. The number of concepts students wrote gradually increased in the exploration phase, and in the concept introduction phase. Before he had learned in this class, he did not write anything. After his experiences in the enzyme kinetics laboratory, his concept map (Figure 4-29a) revealed he knew the nature of enzymes, how they work, and what factors influence enzyme functions. After he had participated in knowledge construction and was introduced the concepts in the lecture class, his concept map (Figure 4-29b) revealed he knew more details of the nature of enzymes, how they work, why some factors influence enzyme function, and how enzymes are useful in everyday life.

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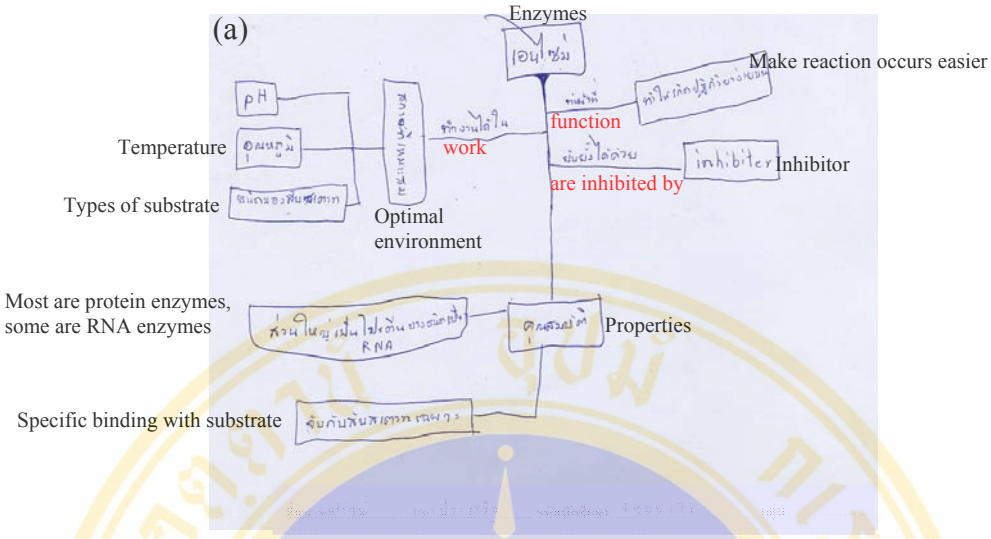
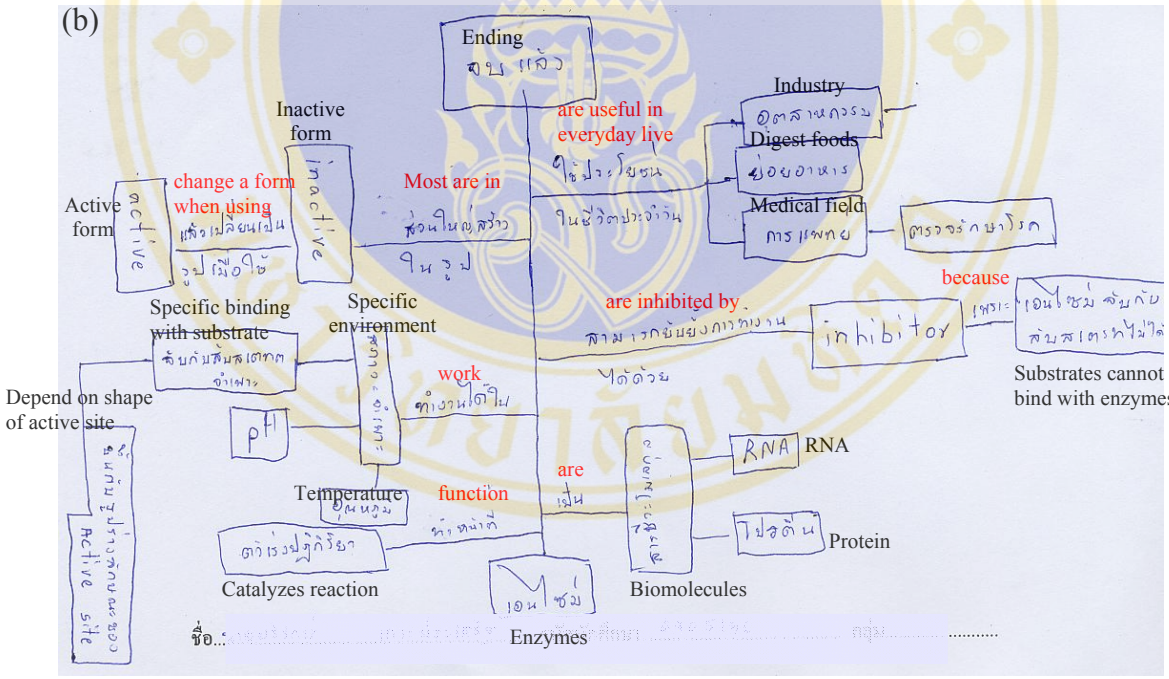


The concept map Songkran sent after his experiences in enzyme kinetics laboratory, he knew the nature of enzymes, how they work, and what factors influence enzyme functions.



The concept map Songkran sent after he was introduced to the concepts in the lecture class. He knew more details of the nature of enzymes, how they work, why some factors influence enzyme function, and how enzymes are useful in everyday life.

Figure 4-29 An example of the concept maps drawn by one student (Songkran) showing his understanding in enzyme kinetics concepts

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The concept maps shown in Figure 4-28 are only one example of students' concept maps that were drawn before and after learning in the class. Although the patterns of students' conceptual growth were similar, the details were different. Some of students' concept maps also revealed some misconceptions about enzyme kinetics. Therefore, the findings from all maps are summarized and presented separately as before and after in order to show the progress of students' knowledge of enzyme kinetics.

Before students had learned in this class, most students mentioned more about the nature of enzymes, some about the factors that affect enzyme functions, and very little about how enzymes work. Their concept maps also revealed some misconceptions about enzyme kinetics:

Enzymes are RNA or nucleic acid. (S095)

Binding between enzyme and substrate must be similar to the situation where a key fits only a particular lock. (S158)

The role of enzymes is food digestion. (i.e., S389)

The reaction which has enzymes produced more products than the reaction without enzyme. (S080)

Enzymes are specific to DNA and RNA. (S371)

Enzymes are made from pancreas or parotids glands. (S132)

After students had experience in enzyme kinetics experiments (exploration phase), they seemed to know more about enzyme functions and the factors that affected enzyme functions. They knew that enzymes speed up the reaction (i.e., S039, S054). Although they knew more factors that affect enzyme functions and explained in detail how these factors affect enzyme functions, students still held misconceptions about the role that enzymes play in digestion (i.e., S086, S380). In addition, other misconceptions were found from their maps drawn at the end of this phase; these are

Inhibitors stop the reaction. (S000)

Low temperatures cause enzymes denature. (S000)

Enzymes make the reactions (that cannot occur themselves) to occur. (S389)

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After they were introduced to the concepts (concept introduction), they seemed to know more concepts of enzyme kinetics. They explained about the nature of enzymes in more details. Most students knew most enzymes are protein, few are RNA. They knew that enzymes have specific properties (i.e. S100). They knew the substrate will bind to the enzyme at the active site but some students still thought that binding between enzyme and substrate must be similar to the situation where a key fits only a particular lock (i.e., S144, S150). They knew the new terms: *binding site*, *catalytic site,* and *allosteric site.* However, some students seemed to be confused about binding site, catalytic site, and the active site. They seemed to think that the binding site and catalytic site are set separately from the active site (i.e., S383, S405). Some thought that the allosteric site is a part of the active site (i.e. S089; S377). In addition, some students seemed to hold the misconception that enzymes react with substrates so that the product is formed (i.e., S098, S137).

Students seemed to know more concepts of how enzymes work in the concept introduction phase. They explained enzymes make the reaction occur faster by a decrease in the free energy of activation, they do not change equilibrium constant and ΔG of the reaction. However, a few concept maps revealed some students held misconceptions that enzymes make reaction occur faster by changing ΔG of the reaction (i.e., S081) or by changing the equilibrium position (i.e., S141). Students knew that enzymes are classified into six classes according to the reaction that they catalyzed. However, some students still thought that enzymes play a role in digestion (i.e., S371). Students knew enzymes can be reused in a particular reaction but some students thought that enzymes do not change (their structure) during the reaction (i.e., S096, S132).

Students also knew more concepts of the factors that affect enzyme functions. They knew different types of inhibitors. Some explained that inhibitor prevent binding of the substrate to the enzyme (i.e., S137). Some students held the misconception that inhibitors stop the reaction (i.e., S000). Some explained that inhibitors cause enzymes to denature (i.e., S093).

Students knew the effect of temperature on enzyme function. Some explained that high temperature causes molecules to move faster. Some explained each enzyme

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works best at its optimal temperature (i.e., S362). Some explained that too high temperature causes enzymes to denature. However, there were students who still thought that all enzymes work well at body temperature (i.e., S081).

Students knew the effect of pH on enzyme functions. Some explained that enzymes will denature at too high or too low pH. However, some thought that enzymes work best at neutral pH (i.e., S081).

Although some students mentioned that the enzyme concentration and substrate concentration affect enzyme functions, they did not explain in details.

4.2.1.5 Summary of Students' Understandings of Enzyme Kinetics

The findings from various tools – ECAT, ELAT, students' work sheets, their assignments, poster, laboratory report, and their concept maps – suggested that students had better understanding of the enzyme kinetics after they had learned in this learning cycle class. Their conceptual understandings of these concepts gradually built during each phase of the learning cycle.

The laboratory activities in the exploration phase not only enhanced students understanding of some concepts (such as the nature of enzymes, how enzymes work, as well as the effect of some factors on the rate of enzyme reactions), but also developed the students' ability to measure and calculate the rate of enzyme reaction. Students also showed the ability to perform an experiment themselves with minimal guidance. They could set an hypothesis, design a control of the experiment, plan the experiment, record data systematically, interpret data, and draw a conclusion based on empirical data. They could also discuss the results appropriately.

The concept introduction phase gave students knowledge of more concepts of enzyme kinetics. This was evidenced from student concept maps. Students not only knew more concepts, they also could make connections between the knowledge they learned in the concept introduction phase and the findings from the experiments in the exploration phase. The evidence of this was found from their work during the concept application phase. Students could use their knowledge to support or discuss the results of their experiments. They could explain the influence of the physical and chemical

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environment on enzyme function more appropriately than they could in the exploration phase.

In the concept application phase, students worked within groups. They showed the ability to use the knowledge they had learned to solve problems or answer the questions related to the concept of enzyme kinetics. They could also explain how enzymes were useful in their everyday lives.

Therefore, promoting students' understanding the concept of enzyme kinetics seemed to be effectively done trough the learning cycle approach. Students' scores on the retention test of ECAT also suggested students did not forget the concepts they had learned in the class.

However, the concepts of how enzymes work were still difficult for students to understand since the proportion of students understanding these concepts was below 50% (see the findings from ECAT). The findings from student concept maps also supported this. The measurement of an initial rate of the enzyme reaction was also difficult for students to understand (see the findings from ELAT and students laboratory work sheets). In addition, some misconceptions were found (see the findings from ECAT, student documents, and their concept maps). The reasons underlying these could be found from other sources of data such as observation data and the analyses of teacher's documents (i.e. laboratory manual, power point, and teaching plan). These will be discussed in the next chapter.

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4.2.2 Students' Perceptions and Their Preferences in the Learning Cycle-based Bromoperxidase Experiment

The students' perceptions and preferences in the learning environment in the learning cycle class and in the traditional class were evaluated using CLES questionnaire and semi-structured interviews. Here, the findings from each measure are presented separately.

4.2.2.1 Findings from CLES Questionnaires

The Constructivist Learning Environment Survey (CLES) questionnaire was used to measure students' perceptions and preferences in the learning environment. This questionnaire was administered to students twice. It was first administered to students before this learning cycle class to obtain their views on the traditional approach used in this biochemistry course (pre-CLES). It was then administered to the students after this learning cycle class to obtain their views on the learning cycle approach (post-CLES). The items of pre-CLES and post-CLES questionnaires produced consistency and acceptable results since the Cronbach alpha reliabilities of these questionnaires were above 0.80 (0.87 for pre-CLES and 0.86 for post-CLES).

The internal reliabilities for each scale of CLES questionnaire ranged from 0.65 to 0.81 (see Table 4-2). Only the scientific uncertainty scale had alpha reliability below than 0.70. However, when item 38 of this scale was removed, this scale produced a better quality of data. Therefore, after item 38 was deleted, each scale of the questionnaire produced consistently moderated and acceptable results (alpha reliability = 0.70).

Table 4-2 Internal reliabilities for each scale of CLES questionnaire

Scales of CLES questionnaire	Items	Alpha reliability
Personal relevance	1, 7, 13, 14, 25, 39 and 37	0.77
Scientific uncertainty	2, 8, 14, 20, 26, 31 and 38	0.65
Critical voice	3, 9, 15, 21, 27, 32 and 39	0.70
Shared control	4, 10, 16, 22, 33, 36 and 40	0.81
Student negotiation	5, 11, 17, 23, 28, 34 and 41	0.79
Attitude	6, 12, 18, 24, 29, 35 and 42	0.70

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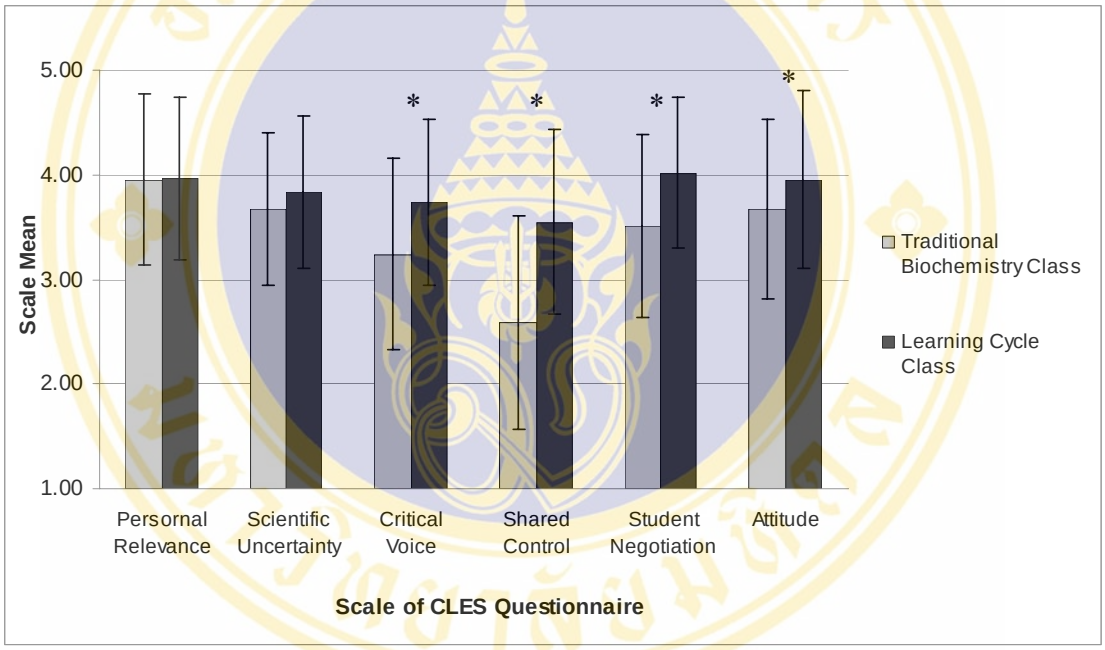
The result as shown in Figure 4-30 presents student responses to pre-CLES and post-CLES questionnaires. It shows that the mean scores for five scales (except personal relevance scale) of the learning cycle class were higher than those of the traditional biochemistry class. These suggested students perceived the learning environment in the learning cycle class as being more constructive than that in the traditional biochemistry class. They also had a higher positive attitude to the learning environment in the learning cycle class than that in the traditional biochemistry class. The details of their responses to each scale were described below.



* Significant difference ($\rho < 0.05$)

Figure 4-30 Second year undergraduate Thai university student's perceptions and preferences in the learning environments of two classes: the traditional biochemistry class and the learning cycle class measuring by using pre- and post-CLES questionnaires (N=72)

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Personal relevance

The mean score of student responses on personal relevance scale for the traditional biochemistry class (3.95 ± 0.82) and for the learning cycle approach (3.96 ± 0.78) were quite similar. This suggested that students perceived the scientific concepts they learned in this biochemistry course, both in the traditional class and in the learning cycle class, were relevant to their lives (see data in Appendix M).

Scientific uncertainty

The mean score of student responses on scientific uncertainty scale were 3.67 ± 0.73 for the tradition approach, and 3.84 ± 0.73 for the learning cycle approach. This difference was not statistically significant. This suggested that before learning in the learning in the learning cycle class, students already perceived that scientific theories are invented by people and this knowledge can change overtime depending on people's values and opinions. Students already know that knowledge can be applied in different ways (see data in Appendix M).

Critical voice

The mean score of student responses on critical voices scale were 3.24 ± 0.92 for the tradition approach, and 3.74 ± 0.79 for the learning cycle approach. This difference was statistically significant. This suggested that the learning cycle approach compared to the traditional approach gave students more opportunities to question the teacher's pedagogical plans and methods and voice their concerns about impediments to their learning (see data in Appendix M).

Shared control

The mean score of student responses on shared control scale equalled 2.59 ± 1.02 for the tradition approach, and 3.55 ± 0.89 for the learning cycle approach. This difference was statistically significant. This suggested that students perceived more shared control in the learning cycle class than in the traditional class. In the traditional biochemistry class, students perceived they had few opportunities to share control with the teacher over the design and management of the learning activities, the

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determination and application of assessment criteria, and the articulation of their own learning goals. However, they perceived the learning cycle class gave them more opportunities to do these (see data in Appendix M).

Student negotiation

The mean score of student responses on student negotiation scale was 3.51 ± 0.87 for the tradition approach, and 4.02 ± 0.72 for the learning cycle approach. This difference was statistically significant. This suggested that students perceived the learning cycle class gave them more opportunities for negotiation than the traditional class. That was, in the learning cycle, they had more opportunities to explain and justify their ideas to other students and to reflect on the viability of their own and other students' ideas (see data in Appendix M).

Attitude

The mean score of student responses on attitude scale was 3.67 ± 0.86 for the tradition approach, and 3.95 ± 0.85) for the learning cycle approach. This difference was statistically significant. This suggested that students had a higher positive attitude to the learning cycle class than to the traditional biochemistry class. That is students preferred the learning environment in this learning cycle class more than that in the traditional class. Students perceived the activities in the learning cycle class were interesting and made them interested in science. They felt they enjoyed learning science in the learning cycle class (see data in Appendix M).

4.2.2.2 Findings from Open-ended Questions of Post-CLES Questionnaire

At the end of post-CLES questionnaire, four open-ended questions were posted in order to obtain information regarding students' preferences and what difficulties or disappointments students may have experienced in the learning cycle class (see questions in Appendix E). An analysis of student responses suggested that all of students who answered the open-ended questions preferred the learning environment used in this study. Although some students had some problems or difficulties, they still

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had a positive attitude toward the learning activities. The details of these are described next.

Upon asking whether they were pleased about the learning environment in the learning cycle class, 52 students stated that they preferred this learning environment. The reasons for their preferences are summarized in Table 4-3. However, 20 students did not respond to this question. From these data, the researcher cannot interpret whether all students participated in this study like or dislike this learning environment. However, the data from other sources – such as interviews and classroom observation – seemed to show that all students liked the learning environment in this learning cycle class.

Table 4-3 Reasons given by 52 second year undergraduate Thai university students for their preferences in the learning environments of the learning cycle class (from student responses to the first open-ended questions in post-CLES questionnaire)

Learning Environment / Student outcomes	Student's reason for preferences	Number of responses
Achievement	The activities in this class let me think more.	1
	I understood why we performed the experiment. We could draw conclusion from the experiment.	2
	The activities in this class made me better understanding (the concepts).	4
	The experiments let me learn the new things.	6
Cooperation	We worked with team to think and to do all activities.	1
	We could share idea.	9
Involvement	This class let me more involvement in teaching and learning.	2
	This class gave us opportunity to design an experiment ourselves.	2
	This class gave me opportunity to present my idea.	4
Relevant to everyday life	I learned something in relevance to my everyday life.	2
Teacher support	The teachers were friendly. They advised us. We could questions.	2
Enjoyable, interesting, and motivation	The activities in this class were interesting. They motivated me to learn.	4
	The experiments were simple, interesting and enjoyable.	14

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4.2.2.2.1 Activities which Students Most Preferred

Only 51 students responded to the question about what activities did they like most. The analysis of their responses suggested that the activities which students most preferred were poster presentation, poster preparation or small group discussion, performing the experiment, and designing the experiment. Some of these students liked more than one activity. The numbers of student's preferences in each activity were summarized in Figure 4-31.

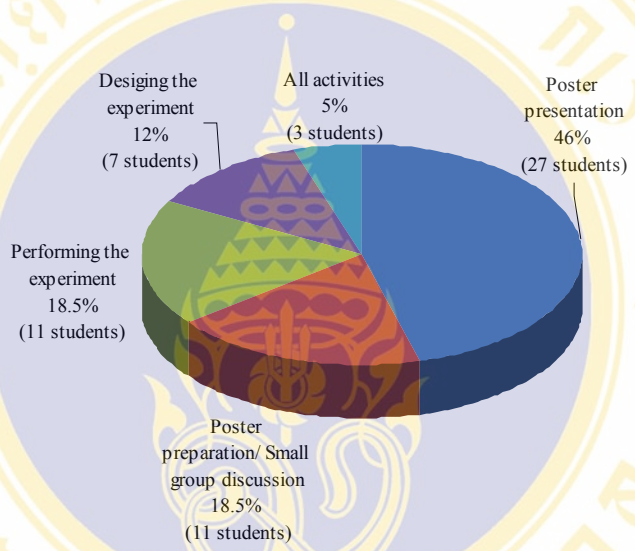

Figure 4-31 The activities in this learning cycle class that 51 second year undergraduate Thai university students most preferred (from student responses to the second open-ended question in post-CLES questionnaire)

Figure 4-31 shows that poster presentation was the activity that students in this class liked most. Students thought that during poster presentation, they could share ideas and discuss their experiment. They had opportunity to exchange their findings. They paid attention to other students' ideas and at the same time they evaluated their own understanding. This not only gave them more knowledge of the concepts, but a better understanding of them. Some students thought they had better understanding when they explained their ideas to another student. Reasons and examples of their statements were summarized in Table 4-4.

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Table 4-4 Reasons and statements given by students who most preferred poster presentation (from student responses to the second open-ended question in post-CLES questionnaire)

Student's reasons	Examples of student's statements
Enjoyable, interesting	I liked poster presentation. I pay attention to our friend's idea. It also was enjoyable and interesting.
Gaining more knowledge	I liked poster presentation. I got more idea from the other students. I paid attention to their experiments.
Evaluating understanding	I liked the activity that our friends present (their works) to us because I could evaluate my understanding of the concepts.
Understanding of concepts	I liked poster presentation. We could question and discuss the results. This made me fun and better understanding the concepts.
	I liked poster presentation. This gave us opportunity to present our idea. I better understood (the concepts) when explained the idea to our friends.
Communication	I like poster presentation because we could show the ability in communication.

Note: Another factor that gives students preferred and enjoyed doing presentation is that teachers in this class were friendly. This was found from student's responses to interview questions which are described in section 4.2.2.3.

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Eleven students in this class preferred the activity where they prepared a poster of the findings from their experiments with other groups. This meant more involvement in the learning activity. They could share and discuss the ideas with peers. They did not only enjoy learning but also had a better understanding of the concepts. Reasons and examples of their statements were summarized in Table 4-5.

Table 4-5 Reasons and statements given by students who most preferred poster preparation/small group discussion (from student responses to the second open-ended question in post-CLES questionnaire)

Student's reason	Example of student's statements
Sharing ideas	I liked group discussion because we could present and share our idea.
Understanding of concept	I liked group poster preparation because every student involved in the activity. This motivated me to join with group. This also gave me a better understanding (of the concepts).
Gaining more knowledge	I liked discussing with our friend because it was not boring. We did not learn seriously. It looked like we were talking but we got more knowledge from this

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Eleven students in this class preferred performing the experiment most. Some of them focused on the experiment they liked, for examples, using an agar slide, investigation the effect of pH on an enzyme reaction, and investigating the effect of sodium azide on an enzyme reaction. The reasons they gave for this activity were that they could see the results; they could draw a conclusion and gain new knowledge from this. They enjoyed doing this with friends. Reasons and examples of their statements were summarized in Table 4-6.

Table 4-6 Reasons and statements given by students who most preferred performing an experiment (from student responses to the second open-ended question in post-CLES questionnaire)

Student's reason	Example of student's statements
Seeing the results	I liked performing the experiment because we could see the results. We could discuss to draw the conclusion.
Gaining new knowledge	I liked performing the experiment because it was new. After doing this, I got new knowledge.
Working with friends	I enjoyed performing the experiment with our friends because it was fun.

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Seven students in this class preferred the activity that gave them the opportunity to design the experiment themselves. They had freedom to plan the experiment. They could share the idea. They thought this gave them good experience working as a team. They enjoyed performing the experiment by following their plan to discover the answers for their questions. They thought they gained more knowledge from this. Reasons and examples of their statements were summarized in Table 4-7.

Table 4-7 Reasons given by students who preferred designing the experiment themselves (from student responses to the second open-ended question in post-CLES questionnaire)

Reason	Example of student's statements
Teamwork	I liked designing the experiment ourselves because everyone in our group involved in this activity. Working with team was efficient.
Involvement	I liked designing the experiment. This gave us opportunity to present our idea.
Discovery	I liked designing the experiment because we could discover the answer for our questions. We could design experiment ourselves. We did not follow only the laboratory manual.
Gaining more knowledge	I liked the experiment because we were free to design the experiment. I enjoyed doing this. This gave me more knowledge.

There were three students who selected more than one activity. All of these students stated they liked all activities in this class because these activities were enjoyable. They stated that *"I like all activities. They were enjoyable"*.

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4.2.2.2.2 Problems or difficulties students had experienced

When students were questioned about the problems or difficulties they had experienced in this learning cycle class, two problems/difficulties were found from the responses of three students. The first problem or difficulty for them was that too many experiments to be reported. The student stated that *"There were too many experiments that we had to report"*. The second problem or difficulty for them was the afternoon lecture, because they were sleepy, learning was difficult. They gave the reason that *"Learning in afternoon lecture was difficult for me since I was sleepy"*

4.2.2.2.3 What things students wanted to change

Only three students responded to the question about what things they wanted to change. One student wanted to change the time for learning in lecture class. He stated that *"We should have lecture class in the morning"*.

Two students wanted to learn another topic using the learning cycle strategy. They stated that *"All topics should be learned like this"*

4.2.2.3 Findings from Student Interviews

At the end of the learning cycle class, 11 students from different student groups in laboratory work were interviewed using semi-structured interview. This gave some examples of how insights from 11 student interviews supported and clarified the findings from questionnaire.

Students had given comments about the learning environment in the learning cycle class and to those in the traditional biochemistry class. These were summarized in Table 4-8.

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Table 4-8 Comments about this learning cycle class and the traditional biochemistry class given by 11 second year undergraduate Thai university students in the interviews

Learning Environment / Student outcome	Student comments: This Learning cycle class	Student comments: Traditional Biochemistry class
Student cohesiveness	Students cited numerous activities of a supportive environment (e.g. designing an experiment, and preparation of a poster), in which peers help each other to find solutions and reason through problems, and show respect for other student's opinions.	Students focused more on dividing work assignments than on working as a collective and supportive team. For example, they divided their responsibility in practical work for each member; one added reagent into test tubes, one measured, one recorded data, and one wrote the laboratory report.
Cooperation	Students felt they could share ideas and work as a team in this class. They felt the activities in this class made them think together, work together, solve and discuss the results together.	Students focused on dividing the work and copying data. Some students helped little in doing group work.
Negotiation	Students felt they had more opportunities to explain their understanding to their friends. They felt they could share results and discuss them with the other groups.	Students felt each group only carried out the experiment to collect the results, and write the laboratory report. They did not share or discuss the results with the other groups.
Share control / involvement / participation	Students felt they had more opportunities to present their ideas. They could design their own experiments. They could complain or question the teachers.	Students thought they could only perform the experiment by following the procedures in a laboratory manual. They did not have opportunity to design their own experiment. They felt they carried out the experiment to collect data for writing the laboratory report.
Teacher support (includes teaching assistant support)	Students felt that teachers (including the teaching assistants) helped them in learning, motivated them to learn, and trained them in the thinking process. The teacher would guide or ask questions, and leave them to their own process. The teacher did not offer answers. The teacher was friendly so they could ask questions.	Students noticed teaching assistants had more involvement in helping them to set up apparatus or teach them to use the instruments. Teaching assistants did not help them to understand the concepts. They could question only one teacher in the laboratory class. This was not enough.

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Table 4.8 (continued) Comments about this learning cycle class and the traditional biochemistry class given by 11 second year undergraduate Thai university students in the interviews

Learning Environment / Student outcome	Student comments: This Learning cycle class	Student comments: Traditional Biochemistry class
Attitudes toward the class	Students thought the inquiry activities in this learning cycle class motivated them to learn. They enjoyed doing the activities. They commented the activities were more interesting and more exciting. One of their reasons was that activities were new for them. Students generally appreciated the freedom in planning their experiments. They enjoyed sharing and discussing with peers. They preferred to explain their understandings or ideas to others. They thought this class gave them an interest in learning science as well as biochemistry.	Students thought this class did not motivate them to learn. They only received knowledge by listening in a lecture. They thought they did an experiment in order to obtain the correct results. This was boring. Sometimes they wondered why they should learn this.
Achievement	Students generally thought that they learned more with inquiry activities in this class. They could explore the experiment in a variety ways instead of looking at things from a single perspective. They gained knowledge from sharing the results and discussing them with peers. They thought they could draw a conclusion from their experiments and had a better understanding when learning in a lecture class. They thought the concepts they learned in lectures could be used to explain their findings from their experiments.	Students noticed they received the knowledge from the teacher in the lecture class. This gave them knowledge of the results they would see in the laboratory class. The experiment showed the real phenomena they had learned in lecture. They thought they learned how to collect the results since they performed the experiment by following the procedure given in a laboratory manual. Sometimes they thought they did not understand what they did in the laboratory class. They would understand this after they wrote the laboratory report.

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Students' comments during interviews as shown in Tables 4-8 suggested the learning cycle class gave students more opportunity to work as a team than the traditional class. In the learning cycle class, students shared and discussed ideas with peers. They perceived they presented their ideas and were involved in the learning activities more than in the traditional class. Students perceived that the teachers in the learning cycle class motivated them to learn and guided them to think more than those in the traditional class. These activities seemed to help them understood the concepts more than only received the knowledge from the teachers. Students also seemed to have more positive attitudes toward the learning cycle approach than the traditional approach. The details of these were described below.

4.2.2.3.1 Students' perceptions of group learning

Students' comments shown in Table 4.7 suggested that group learning seemed to be occurring more in the learning cycle class than in the traditional class. The activities in the learning cycle class, such as designing the experiments, helped them worked as a team. They could share ideas and negotiate with other group members. These seemed to provide ways of helping students to understand the procedural rules in a group experiment. An example of students' statements is as follows:

> This learning strategy in this class [learning cycle class] was good. Everyone worked together, thought together. Normally [traditional class], during the experiment, we divided work – one did experiment, one told how to perform, and one recorded data. In this experiment, everyone had involved in all activities. Everyone knew the experimental procedure. We together did experiment, drew a conclusion, and discussed the results.

Students' responses to the interviews also reflected that during the group activities in this learning cycle class, they shared their results with other groups. They also shared ideas and negotiated their conclusions. This seemed to motivate them to communicate or explain their understanding to others. In addition, the discussion with peers seemed to help them in their understanding of concepts. An example of students' statements is as follows:

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> This laboratory class [in this learning cycle class] was interesting. We worked in groups. We could present the ideas and share ideas. During poster presentation, we compared [the results of] each group. This was interesting. In the others [traditional laboratory class], there were lecture class in the morning and then doing experiment in the afternoon. Each group did the experiment separately. We did not discuss together. After performing the experiment, we were separate. In this class [this learning cycle class], we discussed together. We understood some concepts from our presentation. This encouraged us to think, to communicate, and to explain our understanding.

4.2.2.3.2 Students' perceptions of sharing control with teacher

Students felt the learning cycle class gave them more involvement in the teaching and learning activities than the traditional class. They could present their ideas in this learning cycle class. They stated that *"the activities in this class [this learning cycle class] were good. They gave us more opportunities to present our ideas than the previous classes [traditional]"*.

Students felt the learning cycle gave them more opportunity to design their own experiment than the traditional class. An example of student statements is that:

> This class [this learning cycle class] enhanced my thinking because this class gave us opportunity to design experiment. We could present our ideas. It was enjoyable. Normally we did the experiment by following the direction in the laboratory manual.

Students felt they could complain or question the teacher when they had problems in this class. An example of students' statements is as follows:

> I read the laboratory manual before the laboratory class. It did not give me details. There are many things to do. I did not understand. I questioned the teacher and had an understanding when I designed the experiment. The teacher guided well. I told the teaching assistants that the laboratory manual should give me more details than this. I think we should learn the concepts

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> in the lecture before doing the experiment. If we knew the concept before doing experiment, we would know [what the result should be] so we can set hypothesis correctly.

4.2.2.3.3 Students' perceptions of the teacher's role

Students' comments on the role of the teacher shown in Table 4.7 also suggested that the teachers in the learning cycle class helped them to learn more than those in the traditional class. Students felt that the teacher in this learning cycle class attempted to help them when they had difficulties in class. They stated that *"during the practical work, teachers paid more attention. They guided, explained, and questioned many times. These encouraged us to learn."* In the traditional laboratory class, students felt the teacher help them only when they had problems with instrument or equipment since the teacher already presented the experimental procedure as well as the expected results to them at the beginning of the laboratory class. They stated that *"at the beginning of the laboratory class, teacher explained the laboratory procedures and then let us to perform the experiment to collect data. The teachers did not pay more attention. They only taught us how to use instrument or equipment in the experiment."*

Students perceived that the teachers in this learning cycle class were friendly. They explained that *"the teachers taught well, gave more detail, and were friendly. So the learning did not seem to be serious"*.

Students felt that the teacher enhanced their thinking processes. The teacher did not give them the answers but questioned or guided them. They stated that *"the teachers helped me very much. When we were confused, the teacher and teaching assistants sparked some ideas. They did not give me the answers. They guided me to think"*.

Students felt that in the learning cycle class, especially in the laboratory class, the teachers had more knowledge. They could question the teachers at all times. An example of their statements is as follows:

> The teachers in this class [this learning cycle class] could give me the answers [guided] for every question. Teaching assistants also helped me

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> very much. I could question. Normally [traditional class], teaching assistants only explained how to use the instrument. For laboratory activities, they could not give me the answers. I would questions only one teacher who taught this topic. This was not enough.

4.2.2.3.4 Students' perceptions of their achievements

Students perceived they had a better understanding of the concepts when they had learned in the learning cycle class. Many reasons were given. One reason was that this class gave students experiences before learning of the concepts. Students could see the real results from their experiments. When they learned the concepts in the lecture, they already had an image related to these. An example of students' statements is as follows:

> Doing the experiments before learning in the lecture was helpful. We could see the results of the experiments. Their functions [enzyme function], the results were like this. We had an image. When I learned in the lecture, I had more understanding.

The experiments in this learning cycle class not only allowed students to see the real phenomena but also gave them questions. The concepts they learned in the lecture class could be used to explain these. An example of students' statement is as follows:

> I like to do the experiment like this [experiments in the learning cycle class] because it is easy to understand. It is easier than in the lecture [easy to understand]. If I took notes [notes of what he learned in the lecture], I did not remember. I like this more than that. Doing the experiment before learning in the lecture is helpful. We did it and we had questions [why? question]. We learned in the lecture so we could explain these results.
>
> I like doing the experiments before learning in the lecture. When the experiment failed, something may be wrong. We did not know why. But we could learn and understand this in the lecture. We could question the teacher. If we learn in the lecture before doing the experiment, when we did not understand the experiment, how we do. We learned already but we still did not understand.

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Students thought the learning cycle class gradually gave them the concepts. Their understanding gradually increased through each activity. They could understand the concepts from the experiment and better understand in-depth detail in the lecture. An example of students' statement is as follows:

> I like all days [both laboratory and lecture]. I gradually understood the concepts step by step. We did the experiments. When we did not understand, we questioned the teacher. So we understood. Then we learn in the lecture. We learned in-depth details. When we did not understand, we could question the teacher.

Students thought the whole class discussion also helped them understood the concept. An example of their statement is as follows:

> Whole class discussion showed us how another group worked. Their explanation was easy to understand. This may be because we are the same age. We discuss using same language [that is using their own words during communication] so that it easy to understand.

Students also thought more thinking gave them a better understanding of concepts. They stated that *"I like to learn using this approach more than the previous one [traditional]. This is because the activities in this class made me think more. This gave me a better understanding"*.

4.2.2.3.5 Students' attitude toward the class

Students' responses to the interviews also suggested that students had more positive attitude toward this learning cycle approach than the traditional approach. Students felt the activities in the learning cycle class were enjoyable. An example of their statements is as follows:

> Of all laboratory class I have taken, this laboratory class was enjoyable. I felt that I got many more things than from previous laboratory classes. I felt we worked together as a team in doing the experiment more than in the

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> previous laboratories. Time for doing this (doing the experiment in this learning cycle class), although was long, but I felt time went by very fast. This is because they were enjoyable. I enjoyed.

Students felt the activities in this laboratory class were interesting. An example of their statement is as follows:

> In this laboratory, there were more contents than in the previous laboratories [traditional lab]. For this approach, it's new. I first learned using this approach here. I never learned like this in the other laboratories including chemistry laboratories. This approach is interesting. I think it's good. We did not follow the procedure in the manual directly. We could design the experiment. This showed me that the experiments could have errors. It could fail. How do we know this is correct? We questioned the other groups. However, they did it different from us, so we shared and compared the results. We discussed together. Our results may be O.K [correct].

Students also felt the activities in the learning cycle class were enthusiastic and motivated their thinking process. An example of their statement is as follows:

> The activities in this class were enthusiastic. These were different from the previous classes [traditional lab]. The lecture in this class has cartoon animation. The experiments were enjoyable. There was a group discussion. The activities in this class motivated me to think because we designed an experiment ourselves. I enjoyed doing this. Normally, we did the experiment by following the direction in laboratory manual.

The activities in the laboratory class, particularly designing their own experiment seemed to motivate students to learn in the laboratory class. Students tended to look forward to do the experiment in order to answer their questions. An example of their statement is as follows:

> Today [learning in the learning cycle class] is enjoyable. We could design the experiment. It did not fix the amount of reagent we should use. We did not watch the table in the lab manual to check whether reagent was added

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> right. This [design of experiment], we thought ourselves; we knew how much [reagent] would added.
>
> In our group, we investigated the effects of enzyme concentration [on an enzyme reaction]. At the beginning, we wanted to know that if (the volume of enzyme was) decreased, how the results would be. Then, we really want to knew if [the volume was] too much decreased or increased, what the results would be. So we knew that if we varied it using high range or low range, what kinds of results we got. Doing the experiment like this, we knew by ourselves what we should do next.

Giving students to do the experiment before learning the concept in the lecture seemed to motivate students to learn the concepts in the lecture class. Students seemed to pay more attention in the lecture in order to understand what they did in the experiment. An example of their statement is as follows:

> Learning in this class was enjoyable. We could design the experiments and perform experiment ourselves. We did experiment before learning [of concepts] in the lecture class. I thought doing in laboratory before learning in the lecture had a benefit. That is, if we did not understand in the laboratory, we could ask questions at that time and check again in the lecture. However, if we learn lecture before doing experiment, we had knowledge and knew what we would do [in the experiment], and then we did it. It seemed to be that I did not understand [in the lecture], but I only listened, listened, and feed (concepts) in to my ears. Then I did [experiment]. Umm … it was normally. I was familiar with this for one year. But, today [learning in the learning cycle class] is enjoyable. We could design the experiment. We thought ourselves.

All students who were interviewed seemed to like the learning environment in the learning cycle class and wanted to learn like this in their other classes. However, caution was needed in interpretation of this finding because of the fact that activities in this class were likely perceived by students as more interesting and more exciting. Students had positive responses may probably be because of less stress, and more enjoyment. One student gave interesting statements as follows:

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> I am willing to learn like this approach in the other class. It's interesting. However, it should use this approach alternating with the old approach [traditional]. If use only this approach all times, it may boring. If using this approach alternating with the other approach or new approach, doing experiment will be more interesting. However, I liked this approach more than the old [traditional]. This gave me more thinking so I had a better understanding.

4.2.2.3.6 Students' complaints and suggestions

The disadvantages of this learning cycle approach found from student's responses to interviews is that this approach used more time in designing the experiment than doing the experiment following the procedures in the laboratory manual. However, most students who agreed to participate in interviews thought that learning cycle class was appropriate. It used about the same time as we did in our normal laboratory class.

However, in this learning cycle class, students in each group did not have opportunity to investigate all factors affected enzyme activity. Although they could learn from other groups' presentations, they thought they would have a better understanding if they did all experiments themselves.

One student (who wanted to know the concepts before doing the experiment) complained that the laboratory manual used in this learning cycle class, particularly in enzyme experiment, did not give any detail. She was confused and did not know what she should do. However, most students who responded in the interviews thought that this laboratory manual was suitable. These students thought that the statement was clear and guided them in planning for the experiments.

In this case study, the learning activity began with laboratory in the morning and lecture in the afternoon. Most students complained that the afternoon lecture was not suitable since they were sleepy in the afternoon. They suggested that the lecture class should be in the morning.

Students complained teaching and learning not only in learning cycle class but also in the traditional biochemistry class. They thought that power point that used English language to present the knowledge to them caused problem in their learning.

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However, they though that cartoon animation in the power point helped them had a better understanding. One student stated that:

> When the teacher used English language, I think it's hard to understand. This was not motivating me to learn. I feel that I didn't want to learn in that class. However, this class [this learning cycle class], there were more cartoon animations and the teacher started to teach from the easy one. This was interesting and gave me had a better understanding.

All students who responded in the interviews were willing to learn in other biochemistry topics or other science courses if using this learning cycle approach. Some students suggested that this approach should be used in every biochemistry topic. Some students suggested that learning in biochemistry course should use the learning cycle approach, the traditional approach, as well as new approach alternately. Their reason is that it will make the learning more interesting.

This learning cycle class had four teaching assistants and one main teacher per 72 students. Students thought that it should have more teaching assistants than this. They also suggested other laboratory classes should have the teaching assistants similar to this learning cycle class. They thought that the teaching assistants should be friendly and have more knowledge to guide or answer their questions.

For doing the experiment before learning the concepts, most students interviewed agreed that doing the experiment before learning the concepts made them had a better understanding. They thought this made them think more and they were more able to summarize some concepts related to the concepts they would learn in the lecture. However, many students thought that this approach may be suitable for the topics like enzyme since they had more prior knowledge before learning in this class. In other topics, more information should be given if they have to do the experiment before learning the concepts.

Although almost all students liked to learn using this approach since this gave them a better thinking ability, some students in the interviews suggested that they should learn the concepts before doing the experiment since it would give them had a

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better understanding of the experiment they did and gave them knowledge of what the results should be.

4.2.3 Teachers' Perspectives on the Learning Cycle Approach

The findings from Teacher's Perceptions of Learning Environment Questionnaire (TPLEQ) and the analysis of the documents were used to describe the teachers' perspectives of the effects of the learning cycle approach on their student's learning outcomes. The documents analyzed for this purpose were an article in the newsletter written by one teacher who participated in this study, and the teacher's journal notes of the events during laboratory class. In addition, not only the advantages, disadvantages, and some difficulties the teacher had experienced in using the learning cycle approach, but also some suggestions for using the learning cycle approach in science class derived from their views were described here.

4.2.3.1 Differences between Traditional Laboratory and Inquiry-based Laboratory Teacher had Experiences

The teacher who had experience in teaching biochemistry for more than 20 years and participated in this study wrote an article that reflects the teaching and learning in the traditional laboratory from this experience saying that:

> Previously, undergraduate students and master degree students did the experiment by following the procedures in the laboratory manual….Most students did experiment by using only hand but rarely thinking. We tried to encourage them to read the laboratory manual before coming in the laboratory class by asking them to draw the concept map [of the procedure of that experiment]. However, students normally did group experiment by sharing, like one student hold test tube, another one added reagent into the tube, one told how much to add, and the other one only watched. In preparing the laboratory report, many groups copy file from the previous year. Sometimes they even forgot to delete the name and student ID of the previous one. This is real situation happened in this year, particularly in the laboratory that I had responsibility.
>
> (Source: Teacher's article in the newsletter)

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In this article, she also explained an inquiry-based laboratory she had used in teaching and learning of enzyme kinetics in this learning cycle class:

> We did not start to brief lab like the previous traditional laboratory which start with theory and then left students did the experiment to prove this theory; in this learning cycle students first did the experiment and constructed some knowledge from their own experiment. Then they were given the lecture….In this class, students were given opportunities to design experiment and drew a conclusion themselves. The teacher and teaching assistants only guided and asked the questions to enhance students learning. In one laboratory which had many small experiments, students could select one or two experiments they wanted to investigate. They were freely to design their own experiments by using the provided chemicals and materials. When students finished the experiment, each group that did the same experiments shared results, discussed, and drew a conclusion together, and then prepared a poster to present and discuss with other groups in the whole class. The teachers only gave them more concepts when their explanation was not complete.
>
> (Source: Teacher's article in the newsletter)

4.2.3.2 Teachers Reported the Outcomes of this Learning Cycle Approach

The analysis of the responses on TPLEQ suggested that all three teachers who completed to the questionnaire felt that the learning cycle approach generated better learning outcomes than the traditional approach did. Students in the learning cycle class seemed to demonstrate a greater use of higher-level science process skills, exhibit greater student-student interactions, be generally more manageable and able to design the experiment, generally ask more questions, gain a better understanding of the concepts, show a greater ability to use science process skills, and be generally more eager to learn (see data in Appendix N).

One teacher stated in the article that students in this learning cycle class were able to design the experiments themselves. They could adapt their experiment by using the reagents which had already been prepared (for them). There was a variety of experimental designs. Although their design sometimes did not give good results, and

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their results may differ from the other groups, they could share and discuss until they knew what went wrong. This teacher explained that this situation did not happen in the traditional laboratory where students did the experiment by following the laboratory manual.

The teacher also stated that students in this learning cycle class were able to interpret their results themselves. After students had finished the experiments, they reached conclusions among their peers and discussed before the end of the class. Each group presented their findings to the whole class. Their friends paid much more attention than those occurred in traditional laboratory and asked questions. This was different from the class where teacher discussed the results to the class, in which students did not pay such close attention. In their discussions in this learning cycle class, students communicated in their own style and thus made their friends understood more of their findings. The students also engaged in asking-answering questions among them.

In addition, she stated that giving students opportunities to investigate the experiment before learning the concepts made the teaching of the concepts easier. The students came to the lecture with some ideas and questions to be answered, and they were more familiar some technical termed used although they did not yet know the meaning. As a result, they paid more attention to the lecture.

4.2.3.3 Teacher's Perspectives on the Effectiveness of the Learning Cycle Approach

The researcher's analysis of both the responses to TPLEQ and an article written by the teacher participated in this study suggested that the participants felt that the learning cycle approach was an effective method for teaching science. This approach made the teaching of the scientific concept easier because students already had experiences from their experiments before learning of the concepts and the teacher understood student's misconceptions of the concepts before introducing in depth concepts to them.

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Using the learning cycle approach, one teacher felt that she could identify what may cause the student learning difficulty in understanding the enzyme kinetics. She explained:

> Many students did not understand the basic concepts related to the enzyme kinetics. For example, although students have already learned the concepts of spectroscopic techniques and had experiences that in the laboratory, many students do not seem understand how the spectrophotometer functions. They do not seem to understand what OD or the absorbance means. This causes problems when using this instrument to measure enzyme activity. Students may find it hard to understand what is going on in the reaction.

Her interview response correlated with the journal note of another teacher in student practical work that students did not know the difference between absorbance value and transmittance value. Students did not know what these values mean.

The teachers thought that the learning cycle approach was not only an effective method for teachers in teaching science; it was also an effective method for improving students' reasoning ability. The teachers stated that the learning cycle approach gave students' opportunity to share ideas and to discuss the results of their experiments. It also helped students' ability in thinking processes as students had more opportunities to design their own experiments and to draw a conclusion themselves with only minimal guidance. In addition, the learning cycle approach improved students' ability to communicate with other students because students were given opportunities to share, to discuss, and to present their understandings within groups or in the whole class. These seemed to give students more confidence and more proud in themselves.

The teachers also thought that the learning cycle was an effective method to make students pay more attention in learning because the learning cycle class gave students more involvement in the learning activities. In addition, the teachers felt that this approach is an effective method to give students a better understanding of what they did in the experiments because this approach gave students the opportunity to design and perform the experiment themselves.

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4.2.3.4 Disadvantages and Some Difficulties Teacher found in Using the Learning Cycle Approach

One teacher found that more time was used to help or motivate the students to learn to think, to perform the experiment, and to draw conclusions from their experiment. He found this a disadvantage because this took longer time than letting students performed experiment by following the instruction.

Another found that there needed to be more teaching staff. Teachers had to act as facilitators to help students inquire effectively. If students could not use the instruments or some materials, it may take time in guiding them to design and perform their experiments.

4.2.3.5 Advantages Teachers found in Using the Learning Cycle Approach

The advantages of the learning cycle approach from teachers' perspectives were that this approach (1) allowed students to have more involvement in learning activities; (2) made science more interesting for students; (3) gave students opportunities to present their ideas and to design their own experiments; and (4) offered teachers opportunities to understand student misconceptions related to the topic which would be introduced to them.

4.2.3.6 Teachers' Suggestions for Changes for Further Learning Cycle Classes

The researcher's analysis of teachers' responses to TPLEQ helped the researcher accumulate some suggestions for further learning cycle classes. These included:

(1) the exploration phase of the learning cycle class should have more sources of information related to the concepts students explore;

(2) it should have more teaching assistants who have more knowledge to help students inquire effectively; and

(3) each instrument used in student laboratory class should have the instructions of how to use, this may help students inquire more effectively.

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4.2.4 What Went on in This Learning Cycle Class

This section describes what went on (such as student-student interaction and student-teacher interaction) in the teaching and learning of enzyme kinetics in this learning cycle class. The data triangulation from four sources –VDO camera recording the events during laboratory class, audio recording the conversation during student practical work, teacher's journal notes, and the analysis of the laboratory manual – are used to describe this. The analyses of data from these four sources showed that the teachers visited each student group during their practical work, interacting for a long time, and often taking an active role in students' activities. Some students frequently stopped the teacher to ask questions. The interactions between students and students, as well as students and the teacher varied during each activity.

The activities in teaching and learning of enzyme kinetics topic in this learning cycle class were divided into nine parts: (1) brief laboratory activities in this class, (2) engagement by investigating the bromoperxidase activity in fresh seaweeds by using an agar gel slide containing substrates of enzyme, (3) measuring the enzyme activity by using a simple spectrophotometer, (4) designing and performing an experiment to investigate the effect of chemical and physical environments (pH, enzyme concentration, substrate concentration, inhibitor (sodium azide) on enzyme catalytic reaction, (5) discussing and drawing conclusions from the experiments within a group, (6) sharing the results and discussing them with other groups then preparation of a poster to present the findings, (7) presenting a poster and whole class discussion, (8) learning the concepts in the lecture class, and (9) writing a group laboratory report and answering the questions given in the post-laboratory assignment. The student-student interaction and student-teacher interaction during each activity were described next.

4.2.4.1 Student-Student Interaction and Student-Teacher Interaction before Doing the Experiments

The teacher explained the objectives of the inquiry-based laboratory activities to be performed. Some students paid attention to this, while some did not.

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4.2.4.2 Student-Student Interaction and Student-Teacher Interaction during an Investigation of the Enzyme Activity Using Agar Slides

The students were given agar slide and seaweeds. All students in all groups paid more attention to this activity. There were more interactions between students and the teacher than among students themselves. Some students asked the teaching assistant questions *"What is in the seaweeds?"* and *"What will be happen?"* However, the teacher did not answer their questions but guided them. In the following excerpt, students discussed the result of this investigation with their teacher:

Sritrang:	What is in the seaweeds? What will be happen?
Teacher:	You can observe the results.
Sritrang:	The color of an agar changes from pink to blue.
Teacher:	Does all agar areas change the color?
Banburi:	Only the agar around the seaweeds changes the color.
Teacher:	This agar contains the substrates of an enzyme bromoperoxidase.
Sritrang:	That means the seaweeds contain bromoperoxidase?
Teacher:	Can you explain this to your friends?
Sritrang:	Bromoperoxidase in seaweeds react with substrate in an agar. This causes substrate change to the products.
Teacher:	Good.

After this activity, one student asked question *"How do the scientists know that seaweeds contain enzymes?"* The teacher discussed the matter with students.

4.2.4.3 Student-Student Interaction and Student-Teacher Interaction during Measuring Enzyme Activity

Students measured the absorption at various times of the reaction tube with or without an enzyme. They performed this experiment by following the procedures in the laboratory manual. All members helped each other to do this experiment: one held the test tube, one pipetted the solution into the tube, one read the value from the monitor of a spectrophotometer, and one recorded data. However, only one or two students usually dominated the group during this activity.

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During the measurement of the enzyme activity by using a simple spectrophotometer, students could not used this instrument because the spectrophotometers used in this laboratory class were different from the one they had used. Many students did not know that they should use cuvette (that is special tube used with a spectrophotometer) for that measurement. In addition, they did not know the difference between the absorption value and transmission value. Therefore, the teacher more interacted and discussed with them to solve these problems.

After students finished this experiment, while one student drew a graph, other students paid attention to this and also presented the ideas. From their graph comparing the results of the reactions in the presence and absence enzyme, they could explain that enzyme made the reaction occur faster. The teacher encouraged them to calculate the rate of the enzyme catalytic reaction which was already guided in the laboratory manual. In most groups, one or two students did it while interactions with other students were minimal.

4.2.4.4 Student-Student Interaction and Student-Teacher Interaction during Designing of Experiment

Each group was given an opportunity to select one of the three factors which influenced the enzyme catalytic reaction. The groups which investigated the same factors did experiments near each other and one teacher acted as facilitator. In this activity, the students discussed their own experiment. The questions or statements in the laboratory manual might give them a clue.

In most groups, all group members were equally involved in their project. They worked collaboratively. They first discussed what should be the control of the experiment, what should be varied, and how to do this. During this activity, the teacher visited the groups of students to help and guide them to design their own experiment.

For some groups, they could design the experiment themselves. They only asked the teacher whether their experimental design was correct or not.

For some groups, they seemed to confuse with the volume and concentration. One student asked the teacher about this, and the teacher explained it to them.

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In some experimental designs, the total volume in each test tube was not equal. In the following excerpt, the teacher discussed this with students:

Chongko: Is this plan right?
Teacher: Um…the total volume in each tube is not equal. How would you compare your results?
Chongnang: It's hard to compare because the total volume in each tube is not equal.
Teacher: Could you make them to be same total volume?
Students: ……
Teacher: What should be used to make the total volume of each tube equal?
Moke: Use buffer?
Teahcer: Yeh!

After students performed their experiment, most groups calculated the initial rate of the enzyme catalytic reaction in each variation. They drew a conclusion from their results.

However, some groups did not know how to calculate the initial rate. Then the teacher gave them the meaning of the initial rate and guided them to look at the previous experiment. Thus, they could calculate this and draw a conclusion from their results.

4.2.4.5 Student-Student Interaction and Student-Teacher Interaction during Discussion of the Results with the Other Groups

Each group shared their results with other groups. Students shared data and explained what they did and found in their experiment. Although each group of students did not use the same conditions, most groups got similar results. Some groups although did not get good results, but they could give an explanation for this.

After discussion, they reached the conclusion among their peers and then prepared a poster to present their findings. Most groups discussed how to present their

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results. Some groups selected the best results, some groups put all results together, and some groups used the average of their results.

4.2.4.6 Student-Student Interaction and Student-Teacher Interaction during Poster Presentation

Before poster presentation, teacher and students discussed who would present first. All agreed that the group that had finished the poster preparation first not only should be awarded but also should do the presentation first. They also agreed that the best presentation from popular votes should also receive a reward. During an interview, one student mentioned that *"We want a reward like this, it did not affect us but it makes the learning fun. We did not want a reward like the score, this will make us nervous".*

All students paid attention to the presentation. All groups of students would use all the data for writing report. They also considered themselves part of learning committee. All presenters could report their findings very clearly. Some groups also even demonstrated an ability to use analogy to explain their understanding. One example is that student used lock and key model to explain the binding between enzyme and substrate. They explained that enzyme was similar to key and substrate was similar to the lock. Another example was that student used the hunter who hunted other animals for food to explain the effects of enzyme concentration on enzyme catalytic reaction. They noted that enzyme was similar to the hunter, substrate was similar to animals, and food is similar to product. They explained that *"If there are many hunters, they could hunt animal faster than only one hunter. So they get food faster too"*.

At the end of each group presentation, students asked the presenter questions. All presenters could answer the questions correctly. Each group also helped their presenters to answer the questions. Then, the teacher summarized the findings and explained more for things that were not completed. For example, students showed the results of the effects of substrate concentrations on enzyme catalytic reaction. They did not get a plateau because they used less substrate concentration. The teacher

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showed them the expected results with excess amount of substrate and discussed with them.

4.2.4.7 Student-Student Interaction and Student-Teacher Interaction during the Lecture Class

The afternoon lecture class took three hours. The teacher gave the lecture as in the traditional lecture class but with some interactive lecture. Often times, she asked students' question. The students responded to the question since they already knew the answers or could guess the answer from what they had learned from the laboratory. She introduced the concepts of enzyme kinetics to students by using cartoon animation to present the interaction between enzyme and substrate.

During teaching and learning in this class, many students seemed to pay more attention and some students asked questions. However, some students appeared to be withdrawn from the class.

4.2.4.8 Student-Student Interaction and Student-Teacher Interaction during Writing the Laboratory Report and Answering the Questions in an Assignment

Students were asked to write a group laboratory report by using the knowledge they had learned to explain or support their findings. They were also given a group assignment related to the concepts they had learned in the laboratory and lecture class. However, this situation was not observed since the students done these activities out of the class.

4.3 A Summary of Research Findings

In this study the researcher had implemented the concepts of Lawson's learning cycle based on bromoperoxidase enzyme experiments in teaching and learning of enzyme kinetics in one second year Thai university science class in order to help students conceptualize the kinetics of enzyme reaction.

The findings from ECAT, ELAT, student documents, their concept maps, and their poster presentations suggested that the learning cycle approach based on

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bromoperoxidase enzyme experiments produced good learning outcomes in terms of conceptual understanding. The examination of their laboratory reports also suggested that the leaning cycle approach also helped improve students' science process skills. In their work, it consisted of the following steps: stated the problem, set hypothesis for investigation, observed and recorded data systematically, organized data as a graph that easy to understand, drew a conclusion based on the empirical data, and evaluated the results. Students seemed to have a progress in understanding of enzyme kinetics from the first phase through the third phase of the learning cycle.

The exploration phase allowed students to inquire the laboratory experiments as a team with minimal guidance. They were given opportunities to design and perform their own experiment, to share data, to discuss and reach a conclusion themselves. This laboratory activity not only provided student understanding of some idea of enzyme kinetics, but also developed the students' ability to design and conduct experiments in enzyme kinetics. Students were able to measure and calculate the rate of enzyme catalytic reaction. They were able to draw a conclusion based on their empirical data. Students were able to explain the phenomena by using everyday terms and language. In some cases, students were able to explain their understanding using examples of everyday phenomena or metaphors. These suggested that most students had a better conceptual understanding of some ideas of enzyme kinetics in this phase.

In the concept introduction phase, the depth concepts of enzyme kinetics were introduced to students after they had constructed some knowledge and explained their understanding. This provided students with understanding of both empirical and theory of enzyme kinetics. All students were able to make a connection between the knowledge they had learned and the findings from their experiments. They used the knowledge to support or discuss their findings from the experiments.

The concept application phase gave students opportunities to use the knowledge of enzyme kinetics they had learned to solve or discuss the problem related to these concepts. This provided students with sound understanding of enzyme kinetics for many students and made students had retention of the concepts. This phase also gave some students a better understanding of the concepts.

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Although many students had a better understanding of enzyme kinetics, few students still did not understand some concepts of enzyme kinetics. The findings from the achievement tests as well as student concept maps suggested that the concepts that were considered still being difficult for students were (1) binding between enzyme and substrate, (2) formation of enzyme structure and its active site, (3) role of enzymes and their processing to yield products, (4) how enzymes make a reaction occur faster, (5) rate of enzyme reaction can change over reaction time, (6) why some factors affects rate of enzyme reactions, (7) optimal pH for each enzyme, and (8), influence of temperature on enzyme catalytic reaction.

In addition to conceptual understanding, students' responses to a CLES questionnaire and interviews and data obtained from classroom observation suggested that they preferred this alternative learning method. They enjoyed working in groups. They felt that in this class, they worked more collaboratively than in the traditional class. They were comfortable in learning since teachers were friendly. They had freedom in doing the experiment. As noted in the findings, giving students opportunities to design the experiment seemed to help them gain a better understanding the objective of the laboratory or practical work. They had an understanding what they have done in the experiment and they were able to draw a conclusion based on their data. They were able to construct some knowledge from group discussion after completing the experiments. This way of learning seemed to help them gain a better thinking ability.

Additionally, giving students had experience in the laboratory experiment before introduction of the concepts to them seemed to help teaching of depth concepts become easier due to students had prior knowledge and the teacher already knew what misconceptions student had.

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CHAPTER V
DISCUSSION

Overview

This chapter aims to present the interpretation of the research findings and discuss in relation to other research studies. The implication arisen from this research study is given. The limitation of this research and recommendation for further research are described.

5.1 Scientific Aspects

It needed to be noted here that Thailand closely follows the European universities' science and medicine curricula (Eggins, 2008; Wilson, 1978), thus Thai science major students take biochemistry course in their second year. However, none of these students had done kinetics experiments even in their previous chemistry laboratory classes. They also had never carried out an experiment as collaboratively or with as much emphasis on their own inquiry ability (Prangpatanpon, 1996). This study applied results of the scientific research of this study for teaching/learning in students' enzyme kinetics laboratory. The laboratory designed for students was based on the scientific work on one type of enzyme (bromoperoxidases) from the red seaweed *G. tangi* that has great promise for biotechnological applications. This new enzyme experiment was integrated with the learning cycle – a three-phase inquiry approach consisting of exploration, concept introduction, and concept application – to enhance Thai second year university students' understanding of enzyme kinetics.

In developing the enzyme-based learning cycle in this study, enzyme bromoperoxidase from Thai local natural sources such as red seaweeds *G. tangi* was used for student practical work. Sufficient enzyme can be easily produced for students' practical activity. Using local available materials for teaching and learning is matched with one of the strategies and roadmap of higher education reform that

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emphasizes the knowledge-based society and increasing capacity for self-dependence (MOE, 2004b, Office of the National Economic and Social Development Bord [ONESDB] & World Bank, 2008).

The bromoperoxidase activity from red seaweed *Gracilaria sp.* used as the source of enzyme has been first reported from our research group (Kongkiattikajorn & Pongdam, 2006; Kongkaittikajorn, Ruenwongsa & Panijpan, 2006; Suthiphongchai, Boonsiri & Panijpan, 2008). The specific activity of the crude enzyme from *G. tangi* was found to be 0.1 U/mg proteins which was higher than those of several *Gracilaria* species in Thailand such as *G. changi, G. edulis, G. fisheri* and *G. tenuistipitata*, but lower than those of *G. firma, G. irregularis* and *G. salicornia* (Suthipongchai, 1993). The bromoperoxidase from red seaweed *G. tangi* has been suggested to be a vanadium since its activity was restored by addition of the vanadium into the enzyme. This is not surprising since most bromoperoxidase from marine seaweeds contain vanadium as prosthetic group (Carter-Franklin et al., 2003). The presence of vanadium in the purified bromoeroxidase was established by atomic absorption spectrophotometry and electron paramagnetic resonance. Vanadium in the enzyme probably served to bind two substates (Soedjak & Butler, 1990).

In this study, the properties as well as the assay condition of bromoperoxidase from *G. tangi* were determined before applying to be used in students' laboratory. The pH optimum of the bromoperoxidase from *G. tangi* has been found to be 6.5 which is similar other red seaweed bromoperoxidases which has pH optimum in the range of 4 to 7.5 (Laturnus, Adams & Goméz, 1997; Suthipongchai, 1993).

Concerning the effects of temperature, the bromoperoxidase from *G. tangi* used in this study is not stable, 30% to 92% of the activity lost after incubation at 34^{o}C and 62^{o}C for 5 min, respectively, and was completely lost after keeping at 94^{o}C for 5 min. This is similar to bromoperoxidases from *C. rubrum* and *G. tenuistipitata* which lose their activities after incubation at 50^{o}C to 55^{o}C (Kongkittikajorn & Panijpan, 2006; Krenn, Plat & Wever, 1989). However, bromoperoxidase from *X. parietina* and *S. aureofaciens* are stable at 50^{o}C for more than 2 hours (Wever, de Bore, Krenn & Plat, 1987; Krenn, Plat & Wever, 1988). Bromoperoxidase from *G. fisheri* is also

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thermostable, its activity decreases slightly (about 10%) after incubation at 60°C for 30 minutes (Suthipongchai, 1993).

In the assay of bromoperoxidase activity, this study used a new substrate, bromophenol red (Br_2PR) as organic substrate. Using of Br_2PR as substrate has several advantages over the use of the conventional substrates, monochlorodimedon (MCD) and phenol red. The enzyme assay using MCD produces the colorless halogenated products requires a special instrument as UV-spectrophotometer for detection (Jenwithisuk, 1996). Although the assay using phenol red produces a color product which can be detected by using a simple visible spectrophotometer, but this reaction showed OD change only after a relatively long lag time (Jenwithisuk, 1996). In this study, bromophenol red (Br_2PR) substrate have been shown to produce a better results of a visual conversion of substrate to product. This reaction is novel because bromophenol red has less vacant sites for bromination than that of phenol red so that it is brominated by the enzyme more rapidly when compared to phenol red. This due to the rapidly of the reaction and thus gave intense color in shorter time without lag time.

In the assay of bromoperoxidase activity using bromophenol red (Br_2PR) as organic substrate at optimum pH of 6.5, the reaction can be followed at 590 nm as blue color product (bromophenol blue) is produced. In studying the effect of enzyme concentration, the plot between initial rate vs. enzyme concentrations was found to be linear from a dilution 1/20 (0.01 mg protein) to 1/5 (0.04 mg protein) of crude enzyme (0.1 U/mg), and reach plateau with an increase of enzyme concentration. This result was different from other work using purified enzyme (i.e., Kongkiattikajorn, 1995; Suthiphonchai, 1993). The plateau as occurred in this study is probably caused by higher concentrations of impurities in the more concentrated enzyme extract which act as substrate or reduce the concentration of at least one substrate.

For kinetics study of crude bromoperoxidase, by using the reciprocal plot of the rate and substrate concentrations, K_m values for all substrates were obtained. The K_m values were 31 μM for a newly substrate Br_2PR, 0.33 mM for KBr and 0.15 mM for H_2O_2. Regarding this result, K_m values for halides as KBr was high compared to the K_m for H_2O_2. This is similar to another haloperoxidases reported by many researchers. For example, Wever & Krenn (1990) and Soedjak & Butler (1991) who

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studied the kinetics of V-BrPO (vanadium-bromoperoxidase) in many types of seaweed showed that the K_m values for bromide ranged from 1-24 mM and K_m values for H_2O_2 ranged from 17-92 μM. Almeida et al. (1998, 2001) who studied the kinetics of V-IPO (vanadium-iodoperoxidase) in several seaweeds showed that the K_m values for bromide ranged from 0.82-5.7 mM and K_m values for H_2O_2 ranged from 84-490 μM. Hager et al. (1970) who studied the kinetics of Fe-ClPO (heme-chloroperoxidase) in fungi showed that the K_m for chloride (in pH 3.5) and H_2O_2 were 18 mM and 1.4 mM, respectively. These high values may reflect a low affinity of the active site for halides. In addition, Kongkaittikajorn and Ruenwongsa (2006) have studied the kinetics of V-BrPO and V-BrPO in seaweed G. *tenuistipitata* and showed that for the BrPOI K_m values for bromide and H_2O_2 were $2.17x10^{-4}$ M and $1.0x10^{-4}$ M, respectively. For the BrPOII, the K_m values for bromide and H_2O_2 were $4.72x10^{-5}$ M and $2.47.0x10^{-5}$ M, respectively. The low affinity of haloperoxidases toward halides in marine algae may have evolved in response to an elevated tissue halide concentration, whereas H_2O_2 is maintained at a relatively low concentration (Manley, 2002). Most haloperoxidases were sensitive to high concentration of H_2O_2, for example, the optimum concentration of H_2O_2 for bromoperoxidase from *P. capitatus* was 0.1-0.25 mM and completely inhibited by 2 mM H_2O_2 (Manthey, et al., 1984). The bromoperoxidase of *G. tangi* which has been used in this study was also sensitive to H_2O_2. Its activity dropped at H_2O_2 concentration of more than 0.5 mM. This may be due to competition by the excess H_2O_2 with the other substrates to bind with the enzyme (Everrett et al., 1990).

The activity of bromoperoxidase from *G. tangi* in this study, 50% of the enzyme activity was inhibited by sodium azide (NaN_3). The inhibition by sodium azide has also been found in bromoperoxidase from *C. pilulifera* which was inhibited by 25% at 1 mM azide (Krenn, Izumi, Yamada, & Wever, 1989). Additionally, the crude enzyme from *G. changii, G. fisheri* and *G. tenuistipitata* were inhibited by 90, 60 and 45% inhibition by 0.1 mM azide (Suthipongchai, 1993). The results of this research study suggested that azide functions as non-competition inhibitor with KBr and Br_2PR for the binding sites.

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The results of the scientific research in this study were applied to be used in students' laboratory. Nevertheless, the used of haloperoxidase from red seaweeds *C. corallina* in teaching enzyme, have already been reported by Jervis et al. (2005). This laboratory exercises using haloperoxidase enzymes have been developed for first year to final year undergraduate students within a biosciences scheme. The laboratory designed for the first year students covered the enzyme extraction, enzyme assay, and enzyme kinetics. For the second year students, the laboratory experiments involved using of enzyme as stress biomarker. For the final year students, the laboratory exercise has been focused on enzyme technology as well as environmental technology. Although Jervis et al. have already used haloperoxidase in enzyme kinetics experiment; however, Jervis et al. did not report the students' experimental results as well as their learning achievements. Jervis et al. only mentioned on the used of various substrates to demonstrate the conversion of substrate to yield products by the enzyme. The substrate used for assay haloperoxidase in this study was different from those of Jervis et al., which used monochlorodimedone (MCD) and phenol red (PR) as substrates. As mentioned before, the assay using MCD requires a special instrument as UV-spectrophotometer for detection and thus is not appropriate for student's practical work. In addition, the assay using phenol red presents the problem with long lag time. Bromophenol red (Br_2PR) gave intense color in a short time without lag time. Therefore, Br_2PR is much more suitable to be used as substrate for assay bromoperoxidase activity in students' laboratory experiment.

5.2 Educational Aspects

5.2.1 Implementation of the Enzyme-Based Learning Cycle

Although Jervist et al. (2005) used haloperoxidase in students' laborarory as described above, however, the pedagogical aspects used in this work are also different from that of Jervis et al. (2005). Although Jervis et al. employed the constructivist approach, they did not mention how the teaching/learning was performed in student's laboratory. In this study, the learning cycle approach (Lawson, 1986; 2000) was adapted. This work elaborated the pedagogical aspects (e.g. engagement step, guided

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inquiry-based collaborative learning) to concretely augment the laboratory teaching/learning based on red seaweed bromoperoxidases. The agar gel plates containing Br_2PR for quick surveying of bromoperoxidase activity has been used to engage and motivate students into further investigation of enzyme reaction. The results of this study showed that students' laboratory exercise was aroused by agar plate activity since students all saw the dramatic color development within a short time period. Moreover, the substrate Br_2PR (bromophenol red) was their well known indicator and thus they wanted to learn why and how this indicator can change its color by the enzyme.

The laboratory activities developed for students in this work involved measuring of the enzyme activity and investigating the influence of physical and chemical environment on the enzyme catalytic reaction. The nature of scientific experiment for enzyme reaction in this study is similar to those of several researches, for examples Lin and Lloyd (2006), and Hardee, Montgomery and Jones (2000). This is because the parameters such as pH and concentration of reacting substances are the important concepts in enzyme kinetics that has been widely used in traditional enzyme kinetics experiment. However, the method for conducting the laboratory exercise in this study was entirely different from those of the previous work, which used the laboratory experiment to verify the concepts. In the conventional practical, students were told to carry out the experiment by following the given instructional procedure in a cookbook style (Hardee et al., 2000; Lin & Lloyd, 2006). The newly designed laboratory experiment here was used as vehicles to develop students' concepts on enzyme reaction. It is a shift from laboratory cookbook style to learning cycle-based laboratory. Students were encouraged to design their own experiment and construct their own concept with minimal guidance. Giving students opportunities to actively participate in designing the experiment, as reported in this study, has been shown to produce better outcomes in terms of student motivation in conducting the experiment and understanding of what they have done in their practical work. The students preferred this alternative teaching style and enjoyed planning and carrying out the experiment more than the traditional cookbook style. Students also reported that they had a clearer understanding of what they have been doing in the experiment compared

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to when they were simply given a cookbook list of experimental procedure. In addition, students stated that experiences from such an experiment made them learn the new and in-depth concepts easier.

In this laboratory exercise, since the students were given the chemical reagents at appropriate concentrations and pH, the results on their initial rate vs. substrate or enzyme concentration were generally fair to good. The initial rate measurements with Br_2PR gave linear ΔOD_{590} vs. time. All groups obtained a pH optimum of 6.5 as expected indicating good lab skill. In this laboratory class, the students were encouraged to predict, observe and explain while planning and doing their experiment. In investigating the effects of the enzyme concentrations on the reaction rate, all groups were freely to design their own experiments by using the provided chemicals and materials. Some groups added different amount of enzyme into the assay mixture and made up the total volume with buffer. Some groups diluted the enzyme first and added equal volume of each diluted enzyme to the assay mixture. The plot between initial rates versus enzyme concentrations from some group was linear while some group had plateau depending on enzyme concentration used. However, all groups reached a more or less the same conclusion that the reaction rate or the rate of conversion of substrate to yield product by the enzyme increased with the increase of enzyme concentrations.

Similar to another experiment, the student's experimental designs to investigate the effects of substrate concentration on the initial rate were found to be different among groups. Nevertheless, all groups of students reached a conclusion themselves that an increase in substrate concentrations caused more enzyme-substrate complex formation so that the conversion to yield products was more rapid compared to the reaction with lesser substrate concentration. Although some groups did not get the expected result because they used too little substrate concentrations, they were able to identify what went wrong in their designs after discussing with peers.

As to the effects of NaN_3 on the enzyme activity, the students did not know in the first place that NaN_3 is an inhibitor. Their results showed that addition of NaN_3 inhibited the enzyme activity to a different extent depending upon the concentration of NaN_3 the students used. Their results from one group showed that 5% of the enzyme

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activity remained at 0.5 mM NaN_3 while much less inhibition was observed in some groups due to insufficient amount of NaN_3. However, from this experiment, all students reached a similar conclusion themselves that NaN_3 was an inhibitor for bromoperoxidase. At the end of student's presentations, the teacher also told them the expected results and some added up information to ensure understanding of all students.

Since this was the first time for the students to conduct such an inquiry laboratory as in this learning cycle class, thus the students have shown some hesitation in the very beginning of the exercise. The students carried out their experiments in total collaboration, plotted their results with little prompting from the teaching assistants. The student-centeredness was obvious during their discussion and front-of-the-class presentations during which the teaching assistants were virtually ignored. The data from video and audio recordings showed the body language and verbal exchanges that indicated enthusiasm. This has never been observed in the traditional laboratory where students work as technicians to collect the expected results without thinking (Hofstein & Walberg, 1995; Raths et al., 1986). In the traditional cookbook type laboratory exercise, students divided their tasks without seeing the overall picture and generally can neither discuss nor present their work (Tobin et al., 1994).

It should be emphasized here that this bromoperoxidase experiments was a part of the learning cycle approach in which 3 main phases were involved: exploration, concept introduction and concept application (Lawson, 2001). This approach is contradicts most the general practice in Thailand that laboratory experiment was carried out before the lecture. Given students opportunity to design their own experiment made their learning more meaningful. This helped them gained a better insight into not only on the objectives of the experiment, but also into what they had done in the experiment. Most importantly, they were able to draw conclusions and construct knowledge based on their findings and discussion among peers. This findings support the work of Lawson (1986) that the use of laboratory activities followed by discussion is necessary for concept development. The information exposed to students in their laboratory class raised their questions and sparked their debate and analysis of the reasons for their ideas. The data generated by the laboratory

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therefore can be formulated to a concept. Even though, not all students succeed in construction of their own knowledge but they had a better thinking ability. Another point worth mentioning is that giving students laboratory experience before introducing the concepts made the subsequent teaching of in-depth concepts easier. This is because the students already have some prior knowledge and the teacher knew about students' misconceptions.

To probe students' conceptual understanding, the students were asked to write their own concept maps both before and after the experiment, and after the lecture which took place right after the laboratory. It was found that their concept maps generally improved in terms of more relevant details, better interconnections and descriptions of the links. However, there was some indirect information pertaining more to gene expression and protein synthesis than that of the fully functional enzyme indicating the lack of a certain degree of cogency.

Additionally, there were the much higher scores of post-tests over pre-tests for the Enzyme Conceptual Achievement Test (ECAT). This indicated the success of the designed program. Most importantly, a follow-up test eight weeks after the laboratory showed that students still retained their knowledge (Figure 4-16). These results suggested that many of the students were able to develop conceptual understanding of enzyme kinetics by themselves so that they did not have to memorize as usual and thus the knowledge was retained.

Also there were the higher scores of post-tests over pre-test for the Enzyme Conceptual Achievement Test (ELAT). This indicated students improved in the ability to set hypotheses, design experiments, interpret and draw conclusions. Most students were able to explain what happened in the enzyme catalyzed reaction, how to measure and calculate the initial rate of the enzyme reaction, how to analyze and discuss the effects of chemical and physical environments on enzyme-catalyzed reactions.

Data from analysis of student documents and recorded observations in the laboratory clearly showed that the students had performed a good job to compare, contrast, and discuss their results among peers. Their submitted assignments also revealed their abilities to use the knowledge they had attained to better solve problems

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or answer the questions related to the concepts of enzyme kinetics. They were able to explain the usefulness of enzyme in their daily lives.

The students were very positive about their new experiences as judged from the attitude scale of CLES questionnaire. One of their expressions is the liberation from the bored traditional experiment. Among several activities in the newly laboratory approaches, students discussion in front-of-the-class were done with great enthusiasm and competitiveness. The students paid much attention to the presentation and discussion of their friends with some comments and questions. This reaction hardly occurred in the class with teacher discussion. This was supported by their responses to the questionnaire that they were satisfied with the more active role given to them. This is in accordance with the finding of Fouts and Myers (1992) that the positive attitudes toward learning are often related to achievement and influence it in a positive direction. Such students' achievements and their behavior as appeared in this learning cycle class may result from not only the effectively designed program but also the learning environment. They were free to share and discuss and present their ideas. They felt comfortable in learning because the teacher were friendly and encouraged their participation in the laboratory exercise. The activities were more constructive compared with those of the traditional laboratory class. These were supported by the findings from the Constructivist Learning Environment Survey (CLES) questionnaire. The students responded to the learning cycle class with scores above 3.5 points. This score were higher than the findings from other research works, such as the research of Aldridge, et al. (2000) who investigated constructivist learning environments in Taiwan and Australia. This indicated that the learning activities designed for students in this learning cycle class were more constructive to them. The information exposed to them related to their daily live. The environments of the learning cycle class enable active learning. These findings also confirmed that this learning cycle approach that was shifted from teacher-centered to learner-centered has been designed in appropriate way to serve the need of students.

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5.2.2 Problems in Students' Conceptual Understandings of Enzyme Kinetics

Despite the enhanced leaning ability in several aspects as described above, there were still some students who could not manage to understand some concepts of enzyme kinetics. These concepts included: (1) binding between enzyme and substrate, (2) formation of enzyme structure and its active site, (3) role of enzymes and their processing to yield products, (4) how enzymes make a reaction occur faster, (5) rate of enzyme reaction can change over reaction time, (6) why some factors affects rate of enzyme reactions, (7) optimal pH for enzymes activity, and (8), influence of temperature on enzyme catalytic reaction. These problems are discussed as follows:

Concept 1: Binding between enzyme and substrate

Although students have already learned another model such as induced-fit model that also represented the binding between enzyme and substrate, they still used only lock-key model to explain this phenomena. This may be because lock-key model is simple for them to understand. In this study, most students were able to use a simple model such as lock-key model to explain the binding between enzyme and substrate. Unfortunately, some students have only mentioned that the binding depended on the shapes of an enzyme active site and substrates; they did not mention that binding also depends on the chemical bonds between an enzyme and substrate. The students have been familiar with the lock and key model which lacks the notions of chemical bonding in the binding between enzyme and substrate molecules. Venvilli (2008) has reported that the teachers and textbooks often state, in a very simplified way, that enzymes act in a similar manner to locks and keys. This was supported by the work of Orgill and Bodner (2004) that one of the things that students consistently mentioned was that the teacher should tell their students what they expected the students to understand about the analogy or model they used. Otherwise, students may remember parts of the analogy or model that are not useful. However, if the teacher does make it clear for the purpose of using an analogy or model, it is possible that the students will understand the point of the analogy. The comparison between a lock and key and

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enzyme action is of much greater value if it is developed into a more extended analogy as Venvilli's (2008) explanation:

> Enzymes are made of very large protein molecules that are folded in a way that creates a particular pocket like shape on the surface that exactly fits the shape of the chemicals it reacts with. This pocket is like the hole in a lock that fits exactly the shape of one key and no other keys; in cells this is called an active site. The enzyme is able to hold the chemical reactants in a way that enables the desired chemical reaction to occur much quicker than it would if the enzyme wasn't there. When the chemical reaction has taken place, the products are set free form the enzyme in a similar way to a key that is set free form a lock once it has been opened. The enzyme, like the key, can be reused (p.96).

Orgill and Bodner (2004) found that most students were familiar with the lock and key analogy before they entered their biochemistry classes. They could not understand why their teacher was telling them that the analogy they had learned for so long was not absolutely right. They did not understand that the induced-fit model was supposes to added to their understanding of enzymes and substrates instead of replace their understanding. These were similar to the Thai students involved in this study: they had learned the lock and key model as well as induce-fit model at high school; they still used only the lock and key model to explain the binding between enzyme and substrate. Therefore, the findings as reported by Orgill and Bodner (2004) might be one of the reasons that explained why students in this class did not use the induced-fit model to explain the binding between enzyme and substrate. However, for university students, it is important to emphasize that not all experimental evidences can be adequately explained by using the so-called rigid enzyme model assumed by the lock and key theoretical model. For this reason, a modification called the induced-fit model has been proposed. The induce-fit theory assumes that the substrate plays a role in determining the final shape of the enzyme and that the enzyme is partially flexible. This explains why certain compounds can bind to the enzyme but do not react because the enzyme has been distorted too much. Other molecules may be too small to induce the proper alignment of the active site.

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Concept 2: Formation of enzyme structure and its active site

Although students know that the structure of enzymes and their active sites depend on amino acids functional group of the enzymes, some students cannot explain how each enzyme and its active site are formed. This concept is at molecular level and involves another concept of protein folding.

Enzymes are proteins, which are themselves built up of amino acid residue sequences. The specificity of enzymes is due to their structure, from a specific amino acid sequence to the full three-dimensional globular structure, which in turn controls the nature of the chemical bonds formed, or interaction via intermolecular forces, with the substrates. Most enzymes are globular in shape and the hydrophobic non-polar side-chain groupings tend to align alongside each other towards the centre of the three-dimensional conformation. If not involved in holding the tertiary structure, the hydrophilic polar side-chain groupings tend to be directed toward the outer surface to interface with the aqueous media the enzyme operates in. The amino acid residues, therefore, play an important role in the three-dimensional structure of enzymes and their active sites. An understanding of this concept cannot be gained from the primary sequence of amino acid functional group of the enzyme but necessitates the knowledge of chemical interactions of molecules occurring during enzyme folding. However, this cannot be visualized and therefore it may be reason of why student have difficulties in understanding this concept. The work of Gilbert and Boulter (2000) also supports this finding, they have reported that the lack of spatial ability and visualization among students as problems faced while studying structural chemistry, or enzyme structure in this case.

Concept 3: Role of enzymes and their processing to yield products

Even though, textbooks as well as a lecture class have given students information that enzymes are classified into six types (that are oxido-reductase, trasferase, hydrolase, lyase, isomerase, and ligases) based chiefly on the types of chemical reaction catalyzed, some students still thought that the role of enzymes is digesting or breaking down big molecules to yield small molecules. Some of the students in this study did not understand that breaking down big molecules by enzyme

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meant that the chemical bonds between small subunits of substrate molecules were broken. This suggested that students' problem was probably the lack of understandings of chemical reaction. In addition, some students have thought that enzymes speed up the reaction and do not have an affect on the structure of substrate. This problem may due to students' experience that enzymes make reaction occur faster but the process that enzymes act upon substrate to yield product is still abstract for them.

The findings of Stieff and Wilensky (2003) can be used to support these findings; they stated one reason that causes learning difficulty in chemical reaction (as enzyme reaction in this case) is because the interactions between molecules and atoms occur at a submicroscopic level and cannot be observed directly. This notion is similar to that of Banerjee (1995) who have investigated the conceptual difficulties of undergraduate students in some aspects of chemical concepts and discussed that a particular chemical reaction may be represented with letters or molecular diagrams. In the laboratory, students are further expected to connect the symbolic representations in texts to the macroscopic physical substances used in an experiment. Although the teacher may easily discern the relationships between chemical phenomena at the symbolic, submicroscopic, and macroscopic levels, and represent the phenomena with several representations, students have considerably more difficulty.

Concept 4: How enzymes make a reaction occur faster

Many students in this laboratory class although knew that a reaction occurred faster because of enzyme but they still gave the incorrect reason for this. From students' responses to ECAT, they were confused between thermodynamic quantities (e.g. Gibbs free energy and entropy) and kinetics (e.g. activation energy). This was supported by the work of Cakmakci and Leach (2005) which found that university students did not make a clear distinction between kinetics and thermodynamic ideas. This was because these concepts were often taught separately, and that the relationships or the differences between those concepts were ignored in the curriculum. Thus, students failed to connect between several concepts. Without being explicitly introduced the relationships between some concepts in kinetics and

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thermodynamics, students would have conceptual difficulties in moving between them and resulted in confusion like the students in this study.

Concept 5: Rate of enzyme reaction can change over reaction time

Most participant students understood that the rate of enzyme reaction could change over reaction time, giving the reason that the rate of reaction may gradually decrease with reaction time because enzyme may denature with time or the substrate will gradually decrease. However, some students have thought that the rate of an enzyme catalytic reaction is constant over reaction time, because enzymes are never used up. This finding is similar to those of Cakmakci et al. (2003, 2006), and Waelbroeck (2006). The university students have difficulty in understanding the relationship between reaction rate and time and in explaining how reaction rate changes as the reaction progress. This may due to students' misunderstanding in the definition of enzymes as reflected in their concept maps: enzyme is a catalyst that makes a reaction occur faster and it is unchanged. The work of Van Driel and DeVos (1989b cited in Justi, 2003) also support this misconception. They have found that the brief definitions of catalysis – that focus on the effect of a catalyst on the reaction rate and emphasize that a catalyst is not consumed during the reaction, thus remaining unchanged – could promote misconceptions because students might perceive a catalyst as a magic wand.

Concept 6: Why some factors affects enzyme rate reaction

At the end of the learning cycle class, students seemed to understand the effects of physical and chemical environments on enzyme catalytic reaction; they were able to construct a symbolic representation for relationship between the various conditions and the reaction rate and explained these phenomena correctly. This is in contrast to the findings by Cakmakci et al. (2003, 2006) who have found that although university students could explain that phenomena appropriately, they fail to construct a symbolic representation for relationships between the rate and factors that affect the rate. This may be because students in this study have already constructed a graph of

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their measurement during the practical class, and were able to use that graph to draw a conclusion.

Although the students showed a better performance in providing the appropriate explanation and construct a symbolic representation, a few students according to the retention test, seemed to remember only the pattern of how the chemical and physical environment would change the reaction rate but forgot the reason. This is probably because these students only saw the effects of these factors on the rate of enzyme reaction, but they did not see the process that factors acted on the enzymes. The explanations for these phenomena are at the molecular level and related to the chemical reaction. These explanations were transferred to the students as fact that were being abstract for them. This was supported by the work of Van Driel (2002) that students had problems in providing the explanation in sub-microscopic level on the effects of factors (such as temperature and reactant concentration) on the reaction rate. Although a simple experimental model has been developed to give students visualization of these phenomena in macroscopic level, these concepts were still being difficult for them.

Concept 7: Optimal pH for enzymes activity

In this study, some students still have thought most enzymes work well at pH 7.0 because the pH in the body is neutral and most enzymes operate in the human body. This might be due to the enzyme used in this experiment worked well at pH 6.5; students did not see a variety of enzymes that work well at different pH. Therefore, they may think that the neutral pH is optimal for all enzymes. This was supported by Garratt, Tomlinson, Hardy and Clow (2000). They found that students in their study did not understand the effect of pH on enzyme because to pH change, students could not explore the possible range of effects by studying a single enzyme despite the fact that different enzymes respond differently to pH change.

Concept 8: Influence of temperature on enzyme catalytic reaction

Some of the students do not seem to understand the effects of temperature on enzyme catalytic reaction. Similar to the problem of pH optimum, some students have

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thought enzymes work well at 37^{o}C because this is a body temperature. Moreover, some students have thought enzyme would be denatured at 0^{o}C. In addition, some students seemed to be transferring knowledge from chemical kinetics to enzyme kinetics. They have believed that the rate of enzyme catalytic reaction always increases with temperature because higher temperature makes molecules move faster. These findings are similar to that of Yip (1998) who reported that university students had alternative conception that human enzymes worked best at the body temperature and a drop or rise in body temperature would lead to a loss of enzyme activity and subsequently a breakdown of body functions. Yip have reported that this misconception is reinforced in textbooks and practical manuals. Yip have explained that in order to provide a simplified picture that is comprehensible to students, many textbooks use inaccurate expressions that may lead to misunderstanding, such as 'enzymes work efficiently or best at the body temperature. Several laboratory manuals have recommended incubating enzyme-substrate mixtures at 37°C leading to a misconception that this temperature is optimal for enzyme action. In this study, students did not have opportunity to investigate the effects of temperature on enzyme catalytic reaction and thus still had difficulty in understanding this concept.

5.3 Suggestions for Further Teaching/Learning

As to the applicability of this laboratory for the general audiences, algae with haloperoxidatic activity can be found in tropical and temperate countries, and these algal can be used as source of enzyme in such a laboratory experiment (Kongkiattikajorn & Ruenwongsa, 2006; Suthiphongchai, Boonsiri & Panijpan, 2008; Garcia-Rodriguez, Ohshiro, Aibara, Izumi & Littlechild, 2005).

For the more advanced students, it should be pointed out that the enzyme specificities for halides and peroxides are much stricter than that for the organic or inorganic substrates. Moreover, these enzyme-catalyzed reactions for some organic substrates may not be stereoselective (Carter-Franklin & Butler, 2004; Kamenarska, Taniguchi, Ohsawa, Hiraoka & Itoh, 2007). These haloperoxidases are not typical textbook enzymes.

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Allen and Tanner (2005) stated that the organizational tasks in large-class seemed to be an unmanageable in student laboratory when using active-learning strategy. However, this study has succeeded in implementation of the active learning activities in the large science class. The suggestion here is to adapt laboratory manual from cookbook to a guided-inquiry style, i.e., posting the questions to guide students thinking step by step. In a single 3-hour laboratory class, a strategy to save time and promote teamwork is to break the class into several groups. All groups first practice how to measure the enzyme activity and then each group was responsible for designing the experiment to test only one or two factors (pH, enzyme or substrate concentration, and inhibitor) influencing the rate of reaction. In this procedure the groups that investigated the similar factor, but may be using different design, shared data to interpret of the results, discussed and presented their findings to the whole class. In the context of classroom inquiry, the teachers should not give the direct answer, but facilitate learning to help them inquire effectively, for examples, by asking the question that would guide them to think, providing sources of information to them, and preparing sufficient chemicals and material for student inquiry. The teacher should create learning environments in which students were comfortable in doing the inquiry activities e.g., creating a good relationship between teacher and students and student-student. Research evidences suggested that if students felt comfortable in the learning activities, they would ask question or discuss to the teacher.

Since, the concepts of enzyme kinetics are rather complex. Only the inquiry-based laboratory as the learning cycle may be not enough. It would need integration with other strategies. One feature of enzyme kinetics is that the concepts are mostly at the molecular level. The researcher have found that many students have difficulty in understanding the abstract ideas such as how enzyme acts on substrate to yield products and how enzymes and their active site are formed. Using analogies (Venvilli, 2008) as described above may be helpful. Additionally, visualization tool such as animations may be useful to give an accurate and rich picture of the dynamic nature of molecules and molecular interaction which are often very hard to grasp from the text-based presentations of information (National Science Foundation [NSF], 2001). Williamson and Abraham (1995) who explored the effect of computer animations on

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college science students found that instruction with animations could increase conceptual understanding of by prompting the formation of dynamic mental models of the particulate nature of matter. In this type of instruction, animation may provide a good visual model for representation how enzyme and its active site is formed.

In addition, understanding of enzymes kinetic requires an integrated conceptual understanding of some fundamental ideas such as chemical reaction, and thermodynamics. It would be helpful to emphasize the distinctions between these concepts and to treat closely related concepts of chemistry together. During teaching, the teacher should help students to move back and forth in order to make links between different concepts introduced to them through teaching. The teacher should encourage students to bring together knowledge of different areas and to relate to each other. Without being explicitly introduced the relationships between some concepts, students would have conceptual difficulties in moving between them.

Students experience in enzyme kinetics experiments is very important. Research evidences suggested students could observe the phenomena and construct some knowledge from the experiment (Lawson, 2001). The researcher believes that the experimental activities designed here can be improved for a better outcome. It should give students opportunities to investigate the influence of temperature on enzyme catalytic reaction. To avoid propagating the idea that enzyme works best at body temperature, the teacher should let students carry out investigations to find out the optimal temperature of various enzyme such as salivary amylase and papain. It may bring out the point that the optimal temperatures of the enzymes are variable and usually higher than 37°C. It may be quite surprising or even shocking for the students to find out that the human salivary amylase works well above 50°C (Schneyer, 1951). Such unexpected results provide a cognitive conflict that challenges the students to review their original idea and make appropriate adjustments.

Similarity, the experiment for investigation of optimal pH of different enzymes should also be carried out. The students should have opportunity to find out the optimal pH of various enzyme such as pepsin which performs optimally at pH 2.0 (Voet, Voet & Pratt, 2004), and laccase which works best at pH 4.5 (Lin & Lloyd, 2006).

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However, giving students opportunities to carry out the experiment as mentioned above may not be appropriate for some science class due to high cost of reagent and equipment. Another strategy that may be useful is to give students a secondary data of the enzymes that work best at different pH (or different temperatures) and ask them to analyze and discuss from these data. Leach (2002) found that using the secondary data set help facilitate discussion and also give students understanding some ideas of enzyme kinetics.

5.4 Limitations of the Study

In this study, the researcher has implemented the learning cycle based on bromoperoxidase enzyme experiment in teaching and learning in a Thai university second year science class in order to enhance students' understanding of the difficult concepts in enzyme kinetics. This study did not have the control group because this case study was conducted within an interpretive paradigm.

The biochemistry class in the study involved only 72 students. The small sample size may prevented meaningful statistics analysis and may give less reliability. For example, the reliabilities of data from ECAT and CLES questionnaire although were justified but it was relatively low which may be due to small size of sample. However, the findings here were reliable and justified enough since the researcher used various methods – that were analysis of documents, classroom observation, and interviews) – to triangulate the findings. The small-scale of research in this study appeared to have benefits in terms of rich in-depth information that may be helpful in implication for education.

The teachers involving this study devoted to teach in this laboratory class and also understood the teaching method. They knew about their role in helping student to construct the meaningful concepts. As reported in the findings, the teachers visited students group and discussed with them. They acted as facilitators; they did not transfer knowledge to their students directly, but they gave students questions to enhance them in thinking process, the teachers always guided students during their practical work until the students were able to construct the knowledge from their own experiment. Teachers' understanding of the program contributed a great deal to the

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success of the learning cycle approach. This was supported by the research work of Settlage (2000) who found that teachers' understanding the learning cycle approach influences their abilities to embrace the approach. It is suggested that teacher's guide and teacher's training are needed for the success of this learning cycle approach. In order to transfer these research findings, the teachers should change their style of teaching from not transferring the knowledge directly to students but give students' opportunities to think more and learn actively.

To probe students' retention of the knowledge in this study, the conceptual test was administered to students within two months after this learning unit, which may be short. To ensure that students can conceptualize and not memorize, their knowledge retention should be tested at a longer period of time. The results of this study were obtained from students in the province with moderate grade. The learning outcomes of students from different group with different environment might be different.

Another scientific point to be considered is the source of enzyme bromoperoxidase. The bromoperoxidase from *G. tangi* used in this study was rather unstable; it had to be freshly prepared. It should consider using more stable bromoperoxidase from other species of red seaweed.

5.5 Recommendation for Further Research

1. Similar studies within the same area are needed to confirm the possibility of the effectiveness of the learning cycle approach on students' learning achievement.

2. Replication this study with the similar students in different area of science is needed to confirm the possibility of the effectiveness of the learning cycle approach on students' learning achievement.

3. Replication this study with the larger member of students with different background and a grade is needed to provide more generalized findings on the effectiveness of the learning cycle approach.

4. Additional control group could be included in a future study to ensure that the learning cycle approach is an effective method to enhance students' understanding of enzyme kinetics.

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5. The integration between the learning cycle approach and the other strategies such as using analogies as well as computer simulation is challenging and further studies are needed for fruitful outcomes.

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CHAPTER VI
CONCLUSION

6.1 Scientific Aspects

6.1.1 A simple detection for bromoperoxidase activity from red seaweed using bromophenol red (Br_2PR) as organic substrate has been developed. This reaction gave not only intense color product, but also eliminated problem in lag time usually occurred with phenol red (PR) substrate.

6.1.2 Using the Br_2PR for the assay of bromoperoxidase activity at pH 6.5, the pink color substrate changed to a deep blue color product, which can be followed visually and colorimetrically at 590 nm.

6.1.3 The agar gel plates containing Br_2PR has been developed for surveying bromoperoxidase activity from the seaweeds. The cut seaweeds or fronds were inserted into the agar gel, the change from pink to blue color around the seaweed indicated the enzyme activity.

6.1.4 The colorimetric method for measuring the activity of bromoperoxidase by using Br_2PR as substrate was conducted by following the increase of the brominated product(s) at 590 nm or the decrease of substrate at 430 nm.

6.1.5 Bromoperoxidase from *G. tangi* has been characterized with the following results:

6.1.5.1 pH optimum for the bromoperoxidase activity was at pH 6.5.

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6.1.5.2 The crude enzyme kept at 4°C for 1 day lost 43.6% of its activity. At 62°C, the enzyme lost 98% of its activity.

6.1.5.3 Vanadium pentaoxide (V_2O_5) at concentration 41.67 μM recovered 17% of the crude enzyme after keeping at 4°C for 1 day.

6.1.5.4 Sodium azide (NaN_3) inhibited bromoperoxidase activity. At 1.28 μM NaN_3 inhibited 95% of the enzyme activity. It acted as non-competitive inhibitor with KBr and Br_2PR with K_i 0.07 mM and 0.17 mM, respectively.

6.1.5.5 The rate vs. concentrations of crude bromoperoxidase (0.1 U/mg protein) was linear up to a concentration of 2.7 μg protein/ml and then reach plateau after this concentration (Specific activity = 0.1 U/mg).

6.1.5.6 The K_m values for Br_2PR, KBr and H_2O_2 were found to be $3.1x10^{-5}$ M (pH 6.8), $3.3x10^{-4}$ M and $1.5x10^{-4}$ M, respectively.

6.2 Educational Aspects

6.2.1 The experiment on enzyme (bromoperoxidase) reaction was integrated with a three-phase learning cycle which consists of exploration, concept introduction, and concept application. This learning model was implemented to the second year undergraduate science students and investigated for their effectiveness.

6.2.1.1 ELAT was uses to evaluate student's understanding of the concepts related to enzyme kinetics laboratory, their post-test score (57.68 ± 5.0) was significantly higher than those of the pre-test score (40.10 ± 4.1). Analyses of student's documents (such as laboratory work sheets, a group's laboratory report, and

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poster) supported the findings from ELAT that students had improved skills after conducting the enzyme kinetics experiment.

6.2.1.2 Results from the submitted assignments showed that the students were able to use the knowledge they had learned to better solve problems or answer the questions related to the concepts of enzyme kinetics.

6.2.1.3 The findings from student's concept maps constructed before and after the laboratory exercise, and at the end of the lecture showed improvement in terms of more relevant details, better interconnections and description of the links. Students' post-concept map score (23.01 ± 2.02) was much higher than those of the pre-concept map (4.84 ± 0.95).

6.2.1.4 In using ECAT to evaluate student's understanding of enzyme kinetics covered in the lecture, students' post-test score (36.96±6.71) was significantly higher than those of the pre-test score (25.25 ± 6.46). The students' average score for retention test was found to be 34.23 ± 6.99 which was not significantly different from their post-test score, indicating knowledge retention of the students.

6.2.1.5 In using CLES questionnaire to examine student's perceptions and preference in the learning environment, their scores on all categories of post-CLES were higher than those of pre-CLES. This suggests that students have perceived the learning activities in this learning cycle class as more constructive than those in the traditional class. They were more involving, comfortable, interesting and enjoying in the learning activities due to opportunity to construct their own knowledge. The findings from student interviews also support these findings.

6.2.1.6 In using TPLEQ to obtain teacher's perceptions of the leaning cycle approach, the teachers agreed that this learning cycle promoted the learner-centered atmosphere. They agreed that this is an effective teaching approach to promote students' understanding of scientific concepts.

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6.2.2 It is recommended that this alternative bromoperoxidase laboratory based-learning cycle approach be applied to be used in teaching and learning of enzyme kinetics in another undergraduate science class.

6.2.3 Further studies are needed for fruitful students' learning outcomes.

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APPENDIX

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APPENDIX A
ENZYME CONCEPTUAL ACHIEVEMENT TEST (ECAT)

Objective: To identify second year undergraduate university student's conceptual understanding of enzyme catalyzed reactions

Instructions for Completing this Test:

For each question, please tick √ the box that you think is the best answer, and circle the reason for you choice.

1. **Enzymes are biomolecules.**

   □ **True** □ False □ I don't know the answer

   Reason:

   a. Enzymes are inorganic substances
   b. All enzymes are proteins, and all proteins are enzyme
   c. **Most enzymes are proteins, but some enzymes (such as Ribozyme) are made up of RNA only**
   d. Enzymes may be made up of proteins, lipids, or carbohydrates, depending on the reactants involved in the reaction.

2. **Enzymes influence the reactions of living organisms**

   □ **True** □ False □ I don't know the answer

   Reason:

   a. Enzymes change the environment in living organisms to make them optimal for reactions to occur
   b. Enzymes affect the rate of reaction by speeding up the reaction
   c. Enzymes provide the crucial substrate for the reaction, resulting in product formation
   d. The role of enzymes is in digesting big molecules to make small molecules that are more easily absorbed

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3. Enzymes change substrate molecules to yield products solely by breaking bonds of the substrate molecules.

□ True □ **False** □ I don't know the answer

Reason:

a. The role of enzymes is to break the chemical bonds of the substrate to break down big molecules to yield small molecules

b. Enzymes do not break the chemical bonds of the substrate, but they break down big molecules to yield small molecules, for example, amylase digests starch to yield sugar

c. Enzymes do not affect the structure of substrate at all - instead they only speed up the reaction

d. Enzymes can change substrate molecules to yield products by breaking the chemical bonds of substrate molecules and/or by forming new chemicals bonds in the substrate.

4. Enzymes are specific to a given the reaction.

□ **True** □ False □ I don't know the answer

Reason:

a. Enzymes can speed up <u>all</u> reactions

b. Each enzyme can bind only with <u>one</u> substrate type

c. Enzymes are only active in particular environments

d. Each enzyme has different active sites, so that different enzymes bind with different substrates.

5. An enzyme which digests egg white, cannot digest starch

□ **True** □ False □ I don't know the answer

Reason:

a. Starch molecules are bigger than egg white molecules

b. The enzyme which digests egg white is a digesting protein enzyme, whereas the enzyme which digests starch is a digesting carbohydrate enzyme.

c. Egg white is digested more easily than starch. Egg white and starch are composed of the same small subunits, but the subunits of egg white molecules are connected with chemical bonds, that are different from those in starch

d. The enzyme which digests egg white should digest starch too. However, it cannot digest starch because the environment may not optimal.

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6. **An enzyme will bind only to substrate molecules whose shape exactly matches that of the active site.**

□ **True** □ **False** □ I don't know the answer

Reason:

a. Binding between an enzyme and substrate is similar to the situation where a key fits only a particular lock

b. Binding between enzyme and substrate depends on not only shape, but also on the chemical bonds formed between the enzyme and substrate

c. The shape of the substrate is not important. The reaction can occur if the enzyme and substrate are in an optimal environment

d. Binding between an enzyme and substrate depends on not only their shapes, but also the size of substrate. The substrate must be smaller that the enzyme.

7. **The shape of an enzyme and its active site depends on the amino acid functional group of the enzyme**

□ **True** □ False □ I don't know the answer

Reason:

a. Each enzyme is involved in different reactions, so they have different shapes and active sites

b. The amino acid functional group in an enzyme does not affect the enzyme structure since each enzyme has a specific shape

c. Amino acid functional groups of an enzyme do not affect the enzyme structure because genetic materials determine the shape of an enzyme

d. Enzymes are made up of polymers of amino acids and this determines the structure of the enzyme and its active site.

8. **Enzymes make reactions occur faster**

□ **True** □ False □ I don't know the answer

Reason:

a. Enzymes change the position of equilibrium for a reaction

b. Enzymes decrease the activation energy of a reaction

c. Enzymes increase the rate of molecular movement during reactions

d. Enzymes change ΔG for a reaction, making it more negative.

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9. Vitamins influence the activity of some enzymes

□ **True** □ False □ I don't know the answer

Reason:

a. Vitamins are a type of enzyme

b. Vitamins are cofactors for some enzymes

c. Vitamins affect the activity of all enzymes

d. Vitamins make enzymes stable in changing environments.

10. The rate of enzyme reactions can change over time

□ **True** □ False □ I don't know the answer

Reason:

a. The rate of an enzyme-catalyzed reaction is constant over time, because enzymes are never used up

b. The rate of an enzyme-catalyzed reaction will decrease with time because the enzyme is used during the reaction

c. The rate of an enzyme-catalyzed reaction will decrease with time because the products often inhibit the rate of reaction.

d. The rate of an enzyme-catalyzed reaction **will decrease with time because the substrate will gradually decrease, or the enzyme may denature with time.**

11. When the temperature of a reaction increases, the rate of an enzyme-catalyzed reaction **will always increase**

□ **True** □ False □ I don't know the answer

Reason:

a. Enzymes work well at 37^{o}C because this is body temperature

b. The rate of an enzyme-catalyzed reaction will decrease, since enzymes denature at very high or very low temperatures

c. The rate of enzyme-catalyzed reaction **will decrease at high temperature because the shaped of the enzymes changes at high temperature, meaning substrates cannot bind to the active sites of the enzymes.****

d. The rate of an enzyme-catalyzed reaction **always increases with temperature because higher temperatures make molecules move faster***

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12. Enzymes are denatured at 0^{o}C.

□ **True** □ False □ I don't know the answer

Reason:

a. Most enzymes work normally at 0^{o}C if the pH is neutral

b. Enzymes do not work 0^{o}C because most enzymes are denatured at 0^{o}C.

c. At 0^{o}C, most enzymes are not denatured, instead the amount of enzyme will increase due to replication

d. Most enzymes become inactive or work more slowly at 0^{o}C than at room or body temperature, because chemical reactions are more slowly at lower temperature

13. The enzyme amylase present in the small intestine of humans, works best at normal body temperature

□ **True** □ False □ I don't know the answer

Reason:

a. Amylase works best only in the small intestine irrespective of temperature

b. Amylase will denature at temperatures lower than 37^{o}C

c. The optimal temperature for amylase activity is 37^{o}C in the small intestine, because the activity depends on an optimal balance of pH and temperature

d. Amylase works well in all environments. Acidity, basicity, and temperature do not affect its activity.

14. Acidic and basic (pH) environments influence enzyme activity

□ **True** □ False □ I don't know the answer

Reason:

a. Enzymes are destroyed under conditions of high acidity or basicity

b. Enzymes work well at pH 7.0 because the pH in the body is neutral and most enzymes operate in the human body

c. At low pH (i.e., acidic conditions), enzymes denature, whereas, at high pH (i.e., basic conditions) enzymes are not denatured

d. Changing the pH affects the electric charge of the substrate and of the amino acid functional groups of the enzymes active site, meaning that the enzyme cannot bind to the substrate.

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15. Increasing the amount of substrate does not change the rate of an enzyme catalysed reaction

□ True □ **False** □ I don't know the answer

Reason:

a. The rate of an enzyme catalysed reaction is constant, but adding more enzymes is needed if we want to change all the substrate into products.

b. The rate of an enzyme catalysed reaction decreases as a reaction proceeds, meaning it takes more time to change the substrate into products.

c. When the amount of the substrates increases, the reaction rate of an enzyme catalysed reaction increased until it reaches the highest rate of capacity. After this point the rate of the enzyme catalysed enzyme reaction rate will remain constant

d. The rate of an enzyme catalysed reaction increases with the amount of substrate, meaning the reaction rate will increase, when the amount of substrate increases.

16. In an enzyme-catalyzed reaction, increasing the amount of enzyme results in an increase in the amount of products

□ True □ **False** □ I don't know the answer

Reason:

a. Increasing the amount of the enzyme increases the probability of bond formation between the enzyme and substrate, meaning that more products are formed.

b. Increasing the amount of enzyme results in the formation of product more rapidly, but the amount of product of the reaction does not increase because the amount of substrate is fixed

c. Increasing the amount of the enzyme affects the position of the equilibrium, so that the forward reaction occurs more. Therefore the amount of products increases

d. The amount of products obtained does not depend on the amount of enzyme, but instead it depends on the reaction environment (e.g., pH & temperature)

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17. Inhibitors reduce enzyme activity

□ **True** □ False □ I don't know the answer

Reason:

a. An inhibitor reduces enzyme activity because they cause enzymes to denature

b. An inhibitor binds with the substrate meaning that the enzyme cannot bind with the substrate

c. If an inhibitor binds with the other sites of the enzyme (i.e., not the enzyme active site), it will not affect the enzyme activity

d. An inhibitor may bind to the enzyme active site, or it may bind to the other site of enzyme then change the structure of the enzyme, so that the enzyme cannot bind with the substrate.

18. A substance which has a structure similar to the substrate and can bind with the active site of an enzyme, and will help reaction occur rapidly

□ **True** □ False □ I don't know the answer

Reason:

a. The reaction will occur more rapidly because the total amount of reactants increases

b. The reaction will occur more rapidly because the enzyme can also change the added substance to yield more products

c. The rate of the reaction will not change because the enzyme cannot change the added substance to products

d. The reaction rate will decrease, because the added substance can bind with the active site of the enzyme, reducing the amount of enzyme that can react with the substrate.

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APPENDIX B

ENZYME LABORATORY ACHIEVEMENT TEST (ELAT)

Objective: To identify student's understanding of enzyme kinetics concepts related to practical work.

1. Please read the following statement, and then answer the questions

Ann pipettes some solutions into test-tubes as shown in the following table.

Solution	Tube no. 1	Tube no. 2
1. A solution containing bromophenol red, potassium bromide, and hydrogen peroxide	1.3 ml	1.3 ml
2. A buffer solution	1.7 ml	1.2 ml
3. Bromoperoxidase extracted from marine algae	-	0.5 ml
Total volume	3.0 ml	3.0 ml

These reaction tubes are kept at the room temperature for about 5 min. during which time Ann did not look at the tubes. After 5 min, Ann is surprised when she sees the colour of the solution in tube no. 2 is blue whereas the colour solution in tube no. 1 has not changed and is still red.

1.1 What is the enzyme in this reaction? (maximum 1 point)

Correct answer (1 point):

- Bromoperoxdiase, or
- Bromoperoxidase extracted from marine algae

Alternative conception (0 point)

1.2 What is the substrate for this reaction? (maximum 1 point)

Correct answer (1 point):

- Bromophenol red, potassium bromide, and hydrogen peroxide

Partially correct answer (0.75 point):

- Bromophenol red (main substrate)

Partially correct answer (0.5 point):

- Potassium bromide and/or hydrogen peroxide

Alternative conception (0 point)

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1.3 Why does tube no. 1 have more buffer solution than tube 2? (maximum 1 point)

Correct answer (1 point):

- Tube A is used as **a control**, it has additional buffer **instead of the enzyme solution**

Partially correct answer (0.5 point):

- To adjust the total volume of the solution in each tube to be equal

Alternative conception (0 point)

1.4 What hypothesis could be used to test this experiment? (maximum 1 point)

Correct answer (1 point):

- The enzyme affects the rate of the chemical reaction, whatever change occurs in the tube containing the enzyme will differ from the tube without the enzyme.
- The reaction containing the enzyme will give different results to the reaction without the enzyme.

Partially correct answer (0.5 point):

- The enzyme influences the chemical reaction.

Alternative conception (0 point)

1.5 The blue colour in the solution is due to what substance? (maximum 1 point)

Correct answer (1 point):

- Product of the reaction, or
- Bromophenol blue

Alternative conception (0 point)

1.6 What conclusion do you think Ann could make from the results of this experiment? (maximum 1 points)

Correct answer (1 point):

- The bromopeoxidase causes the bromophenol red to change since a new substance has been formed
- The enzyme causes changes to the substrates to yield products

Partially correct answer (0.5 point):

- The enzyme affects the chemical reaction

Alternative conception (0 point)

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1.7 Write the chemical equation for the reaction that occurs in tube no. 2 (maximum 1 point)

Correct answer (1 point):

Enzyme + Substrate ⟶ Enzyme + Product

or

Bromophenol red + Potassium bromide + Hydrogen peroxide $\xrightarrow{\text{BPO}}$ Bromophenol blue

or

Bromophenol red + Potassium bromide + Hydrogen peroxide $\xrightarrow{\text{BPO}}$ Blue colored product

or

Bromphenol red (pink colour) — Bromphenol blue (blue colour)

Partially correct answer (0.5 points):

Enzyme + Substrate ⟶ Product

Alternative conception (0 point)

2. If you want to decide 'does the temperature affect the rate of an enzyme-catalyzed reaction?":

2.1 In a control experiment, list at least 2 of the controlled could be used (maximum 1 point)

Correct answer (1 point):

- Answer two of:
  - enzyme concentration
  - substrate concentration
  - buffer pH
  - enzyme should be same
  - type of buffer
  - buffer concentration

Partially correct answer (0.5 point):

- One of the above answers

Alternative conception (0 point)

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2.2 What would be the independent variable for your experiment? (maximum 1 point)

Correct answer (1 point):

- Temperature

Alternative conception (0 point)

2.3 How could you follow what happens in this experiment? (maximum 2 points)

Correct answer (2 points):

- **Measure the change in absorbance against changes in temperature** at the same fixed wavelength, then plot a graph of absorbance versus time. Calculate the rate of the enzyme catalyzed reaction from the slope of graph, or
- **Measure the increase in product concentration or the decrease in substrate concentration** with time by measuring the absorbance at time intervals at fix wavelength, then plot a graph of product versus time. Calculate the rate of the enzyme catalyzed reaction from slope of graph, or
- **Observe the changing of color of solution** after adding enzyme into the reaction (the enzyme should be added in each reaction at the same point in time).

Partially correct answer (1 points):

- One of the highlight above answers

Partially correct answer (0.5 point)

- Using spectrophotometer, or
- Using an agar slide for assay enzyme activity

Alternative conception (0 point)

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3. Joe did an experiment in which he measured the enzyme activity. He added enzyme solution into a tube containing a substrate solution, mixed it and started a timer. He measured the increase in product concentration of this reaction by measure its absorbance every 10 sec. for 1 min. His results are shown in the following table.

Reaction time (sec.)	0	10	20	30	40	50	60
Product (mg/ml)	0.00	0.25	0.5	0.75	0.80	0.85	0.85

3.1 Calculate the initial rate of this reaction (maximum 1 point)

Correct answer (1 point):

- 0.25 mg/ml per of protein per second

Partially correct answer (0.5 point):

- 0.25

Alternative conception (0 point)

3.2 Calculate the reaction rate at 50 to 55 seconds? (maximum 1 point)

Correct answer (1 point):

- 0 mg/ml per of protein per second

Partial correct answer (0.5 point):

- 0

Alternative conception (0 point)

3.3 Why the rate at 55 seconds differ from the initial rate? (maximum 1 point)

Correct answer (1 point):

- Because all the substrate has already changed to product
- Because the amount of the substrate is limited, all is changed

Partial correct answer (0.5 point):

- Because the concentration of product does not increase

Alternative conception (0 point)

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3.4 If this enzyme is boiled for 10 min. before doing this experiment, what effect would this have on product formation? (maximum 1 point) and Why? (maximum 1 point)

- Correct answer (1 point):
  - No product is produced
  - The product would be produced more slowly
  - The rate of product formation is lower than that for the reaction using non-boiled enzyme

  Alternative conception (0 point)
- Correct reason (1 point):
  - The high temperature causes the enzyme to denature

  Partially correct reason (0.5 point):
  - The temperature affects the enzyme activity

  Alternative conception (0 point)

3.5 Other than the temperature, what other factors affect the rate of enzyme catalyzed reactions? List only 1 factor and explain how it influences the rate point) and say why (maximum 3 points).

Any of the following factors is scored 1 point for a maximum of 3 point.

- pH (1 point)

  Enzymes work best or have the maximum effect at a particular, optimum pH (1 point). At pH that higher or lower than the optimum pH, the activity or rate of reaction decreases. This is because **acidic or basic solutions affect the structure of substrate and of the enzyme active site (1 point),** meaning that the enzyme cannot bind to the substrate at that pH.
- Substrate concentration (or the amount of substrate) (1 point)

  When the amount of the **substrates increases, the reaction rate of an enzyme catalysed reaction increases (1 point)** until it reaches the highest rate of rate because **this increases the chance of the ES-complex formation (1 point).** After this point the rate of the enzyme catalysed enzyme reaction rate will remain constant because all enzymes are go via a ES-complex.
- Enzyme concentration (or the amount of enzyme) (1 point)

  When the amount of the **enzyme increases, the reaction rate of an enzyme catalysed reaction increases (1 point)** until it reaches the highest rate of reaction because **the amount or concentration of the enzyme increases the chance of formation of the ES-complex (1 point)**. After this point the rate of the enzyme

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catalysed enzyme reaction will remain constant because the substrate becomes limiting in the formation of the ES-complex.

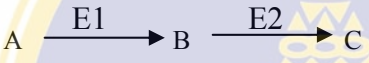
- Activator (1 point)

  An activator **causes the reaction to go faster (1 point)** since **it stabilizes the binding between the enzyme and substrate (1 point)**.

- Inhibitor (1 point)

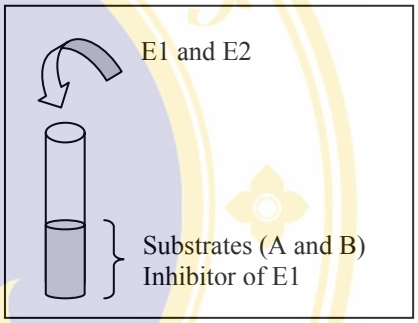
  An inhibitor **reduces the rate of enzyme reaction** and thus the enzyme activity (1 point) because **it prevents or inhibits the binding of the substrate to the enzyme active site** (1 point)

4. Consider the following reaction



Where

A is substrate of enzyme E1,
B is substrate of enzyme E2



What will happen if an excess of inhibitor of E1 is added to the reaction before E1 and E2 are added? (select one of the following answers then give your reason) (2 points)

A. The concentration of C produced increases because ……

B. The concentration of C produced gradually decreases because ……

C. C is not produced (1 points).

This is because <u>B is not produce</u> so that <u>E2 does not have substrate</u> to yield product, C (one of the underline reasons is given 1 point).

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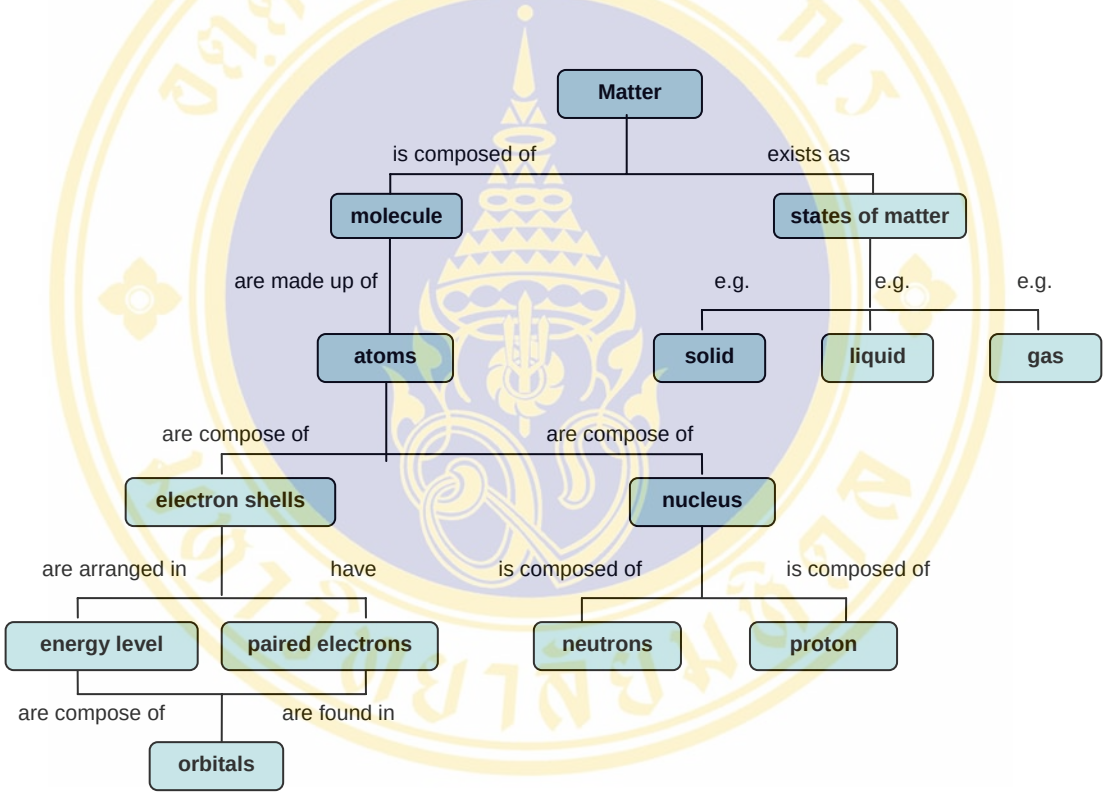
APPENDIX C
EXAMPLE OF CONCEPT MAP - MATTER

Objective: To give students idea of using the words to link between the concepts in order to represent the understanding of the concepts



Reference

Gills, V.R., & McaDougall, G. (2007). Reading to learn science as an active process. *The Science Teacher, 74*(5), 45-50.

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APPENDIX D

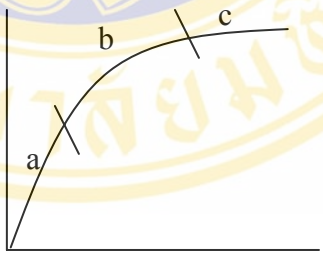
POST-LABORATORY ASSIGNMENT

Objectives: To give student opportunities to use or apply the knowledge they learned in laboratory and lecture classes in to new situation, and to identify students' conceptual understanding in enzyme kinetics.

1. In the reaction which has enzyme as catalyst, how do you do to make more products in the short time (unlimited money)?

2. Do all enzymes work well at the same environment? Explain and give an example.

3. From the experiment, we draw graph to represent the relationship between the absorbance and time as the following picture:



Answer the following questions:

3.1 Where the area of the graph (a, b, or c) has the highest rate?

3.2 Where the area of the graph (a, b, or c) has the lowest rate?

3.3 Where the area of the graph (a, b, or c) should be used to study the effect of physical or chemical environments on enzyme-catalyzed reaction?

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4. In measuring the progress of the product formation (in the reaction which has enzyme as catalyst), you found that the reaction rate increases at the first time interval. After that the reaction rate is constant. Explain why.

5. In measuring the rate of enzyme-catalyzed reaction when you increase the amount of substrate, you found that the reaction rate increases. However, after one point, the rate does not increase even though you add more substrate. Explain why.

6. Are the inhibitors useful? How? Give an example.

7. When you have fever (too high body temperature), is this affect the reaction in your body? Explain.

8. Which commercial products in your local supermarket compose of the enzyme? Give one example and explain the application of the enzyme in that

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APPENDIX E

CONSTRUCTIVIST LEARNING ENVIRONMENT SURVEY (CLES) QUESTIONNAIRE

Pre-CLES Questionnaire

Objective: To determine student perceptions of and preferences in the learning environment in traditional biochemistry class.

Section 1. Personal Information

Gender □ Male □ Female

Student ID ……..Level …..

Major ……..

Section 2. Teaching and Learning Environment in General Biochemistry Class

In the general biochemistry class…	Almost Always	Often	Sometimes	Seldom	Almost Never
1. I learn about the world in relevance to my life.					
2. I learn that scientific theories are human inventions.					
3. I can ask the teacher "Why do we have to learn this?"					
4. I help the teacher to plan what I'm going to learn.					
5. I get the chance to share ideas with other students.					
6. I look forward to the learning activities.					
7. New learning starts with problems about the world in relevance to my life.					
8. I learn that science is influenced by people's values and opinions.					
9. I feel free to question the way I'm being taught.					
10. I help the teacher to decide how well my learning is going.					
11. I can talk with other students about how to solve problems.					
12. The activities used in this class are interesting.					
13. I learn that how science can be part of my life.					

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In the general biochemistry class…	Almost Always	Often	Sometimes	Seldom	Almost Never
14. I learn that the views of science have changed over time.					
15. I can complain about the activities that are confusing.					
16. I have a say in deciding the rules for classroom discussion.					
17. I try to make sense of other students' ideas.					
18. The activities in this class make me interested in science.					
19. The concepts learned in this class help me to better understand the world in relevance to my life.					
20. I learn that the biochemical knowledge can be applied to be used in different ways.					
21. I feel free to tell the teacher about anything that stops me from learning.					
22. I have a say in designing how much time I spend on an activity.					
23. I ask other students to explain their ideas.					
24. I enjoy the learning activities in this class.					
25. I learn interesting things about the world related to my life.					
26. I learn that scientific knowledge can be questioned.					
27. I'm free to express my opinion.					
28. Other students ask me to explain my ideas.					
29. I am confused about the concepts learned in this class.					
30. What I learn in this class has no relevance to my life.					
31. I learn that science reveals the secrets of nature.					
32. I can express my opinion.					
33. I have a say in deciding what will be done on the test or evaluation.					
34. Other students explain their ideas to me.					
35. The learning activities in this class are a waste of time.					
36. I have a say in deciding what activities I do.					
37. What I learn in this class has no relationship to my life.					
38. I learn that scientific knowledge is beyond doubt.					
39. I feel unable to complain about anything.					
40. I have a say in how my learning is assessed.					
41. Other students pay attention to my ideas.					
42. I feel tense about what is occurring in this class.					

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Post-CLES Questionnaire

Objective: To determine student perceptions of and preferences in the learning environment in the learning cycle class based on bromoperoxidase enzyme experiments.

Section 1. Personal Information

Gender □ Male □ Female

Student ID ……..Level …..

Major ……..

Section 2. Teaching and Learning Environment in Enzyme Kinetics Topic

In this class	Almost Always	Often	Sometimes	Seldom	Almost Never
1. I learn about the world in relevance to my life.					
2. I learn that scientific theories are human inventions.					
3. I can ask the teacher "Why do we have to learn this?"					
4. I help the teacher to plan what I'm going to learn.					
5. I get the chance to share ideas with other students.					
6. I look forward to the learning activities.					
7. New learning starts with problems about the world in relevance to my life.					
8. I learn that science is influenced by people's values and opinions.					
9. I feel free to question the way I'm being taught.					
10. I help the teacher to decide how well my learning is going.					
11. I can talk with other students about how to solve problems.					
12. The activities used in this class are interesting.					
13. I learn that how science can be part of my life.					
14. I learn that the views of science have changed over time.					
15. I can complain about the activities that are confusing.					
16. I have a say in deciding the rules for classroom discussion.					
17. I try to make sense of other students' ideas.					
18. The activities in this class make me interested in science.					

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In this class	Almost Always	Often	Sometimes	Seldom	Almost Never
19. The concepts learned in this class help me to better understand the world in relevance to my life.					
20. I learn that the biochemical knowledge can be applied to be used in different ways.					
21. I feel free to tell the teacher about anything that stops me from learning.					
22. I have a say in designing how much time I spend on an activity.					
23. I ask other students to explain their ideas.					
24. I enjoy the learning activities in this class.					
25. I learn interesting things about the world related to my life.					
26. I learn that scientific knowledge can be questioned.					
27. I'm free to express my opinion.					
28. Other students ask me to explain my ideas.					
29. I am confused about the concepts learned in this class.					
30. What I learn in this class has no relevance to my life.					
31. I learn that science reveals the secrets of nature.					
32. I can express my opinion.					
33. I have a say in deciding what will be done on the test or evaluation.					
34. Other students explain their ideas to me.					
35. The learning activities in this class are a waste of time.					
36. I have a say in deciding what activities I do.					
37. What I learn in this class has no relationship to my life.					
38. I learn that scientific knowledge is beyond doubt.					
39. I feel unable to complain about anything.					
40. I have a say in how my learning is assessed.					
41. Other students pay attention to my ideas.					
42. I feel tense about what is occurring in this class.					

Please answer the questions in next page.

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Please be brief, but thorough, in answering the following questions:

1. Are you pleased about the activities used in the practical class? Why? Why not?

   ……

   ……

2. What activities do you like most in the practical class? Why?

   ……

   ……

3. Do you have any problem(s) in the class or anything that is difficult for you to do? Please explain.

   ……

   ……

4. What thing (s) that you want to change? Why?

   ……

   ……

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APPENDIX F
SCORING GUIDELINES FOR THE CONSTRUCTIVIST LEARNING ENVIRONMENT SURVEY

This instrument consists of both of both positive and negative statements which students must answer on a scale that ranges from "almost always" to "almost never" For **positive** item statements, the "almost always" choice would receive a 5 moving on down to the "almost never" choice which would receive a 1. For **negative** item statements, the numbering procedure is reversed (Salish I Program 1997)

Example:

In this class…	Almost Always	Often	Sometimes	Seldom	Almost Never
(+) 1. I learn the world related to my life.	5	X 4	3	2	1
(-) 37. What I learn in this class does not involve in my life.	1	X 2	3	4	5

Sample item 1 would be scored as a 4 while sample in item 37 would be scored as a 2. The total score would be 4+2 = 6, in this example.

I. Personal Relevance Scale (PR)

This scale is concerned with students' experience of the personal relevance of school science. The scale has been designed to measure the extent to which student perceives the relevance of school science to their out-of school lives.

From a constructivist perspective, the classroom environment should not promote a discontinuity between school science and students' out-of-school lives by evoking an abstract and decontextualized image of science. Rather, the classroom environment should engage students in opportunities to experience the relevance of school science to their everyday interests and activities and to use their everyday

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experiences as a meaningful context for the development of their formal scientific knowledge.

Items: 1. (+) 25. (+)
7. (+) 30. (-)
13. (+) 37. (-)
19. (+)

II. Science Uncertainty Scale (SU)

This scale is concern with students' perceptions of science as a fallible human activity. The scale has been designed to measure to extent to which students perceive science to be an uncertain and evolving activity embedded in a cultural context and embodying human values and interests.

From a constructivist perspective, the classroom environment should not promote a scientist view of science as a supreme universal mono-cultural activity that is independent of human interests and values or promote the objectivist myth that science provides an accurate and certain representation of objective reality (such as a correspondent theory of truth). Rather, the classroom environment should be concerned with engaging students in opportunities to learn to be sceptical and critical about the nature and value of science. In particular, to learn that scientific knowledge is evolving and provisional. It is shaped by social and cultural influences and arises from human interests and values.

Items: 2. (+) 26. (+)
8. (+) 31. (-)
14. (+) 38. (-)
20. (+)

III. Critical Voice Scale (CV)

This scale is concerned with students' development as autonomous learners. In particular, the scale has been designed to measure students' perceptions of the extent to which they are able to exercise legitimately a critical voice about the quality of their activities.

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From a constructivist perspective, the classroom environment should not favour technical curriculum interest (such as covering the curriculum content) to an extent that accountability for classroom activities is directed largely towards an external authority. Rather, the teacher should be willing to demonstrate his/her accountability to the class by fostering students' critical attitudes towards the teaching and learning activities. This can be achieved by creating a social climate in which students feel that it is legitimate and beneficial to question the teachers' pedagogical plans and methods and to express concerns about any impediments to their learning.

Items: 3. (+) 27. (+)
9. (+) 32. (+)
15. (+) 39. (-)
21. (+)

IV. Shared Control Scale (SC)

This scale is concerned with another important aspect of the development of student autonomy, namely students sharing with their teachers' control of the classroom learning environment. In particular, the scale has been designed to measure students' perceptions of the extent to which the teacher involves them in the management of the classroom learning environment.

From constructivist perspective, students should not be required to adopt the traditional role of compliant recipients of a predetermined pedagogy that is controlled entirely by the teacher. Rather, the teacher should invite students to share control of important aspects of their learning by providing opportunities for them to participate in the process of designing and managing their own learning activities, determining and applying assessment criteria, and negotiating the social norms of the classroom.

Items: 4. (+) 33. (+)
10. (+) 36. (+)
16. (+) 40. (+)
22. (+)

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V. Student Negotiation Scale (SN)

This scale is concerned with negotiation among students. The scale has been designed to measure students' perceptions of the extent to which they interact verbally with other students for the purpose of building their scientific knowledge within the consensual domain of the classroom.

From constructivist perspective, the classroom environment should not require students to learn in social isolation from other students or to regard the teacher or textbook as the main arbiter of what counts as viable scientific knowledge. Rather, the classroom environment should be concerned with engaging students in opportunities to explain and justify their newly developing ideas to other students, to make sense of other students' ideas and reflect on the viability of their ideas, and to reflect critically on the viability of their own ideas.

Items: 5. (+) 28. (+)
11. (+) 34. (+)
17. (+) 41. (+)
23. (+)

VI. Attitude Scale (AT)

This scale has been included to provide a measure of the concurrent validity of the CLES. The attitude scale has been used extensively in research on science laboratory classes, and has an established reliability. The scale measure student attitudes to important aspects of the classroom environment, including their anticipation to the activities, their sense of worthwhileness of the activities, and the impact of the activities on student interest, enjoyment and understanding.

Items: 6. (+) 29. (-)
12. (+) 35. (-)
18. (+) 42. (-)
24. (+)

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APPENDIX G

TEACHER'S PERCEPTION OF LEARNING ENVIRONMENT QUESTIONNAIRE (TPLEQ)

Objective: To obtain information regarding the teacher's view of the learning environment in the inquiry-based learning cycle class

Section1. Personal information

Position □ Teacher □ Teaching assistant

Gender □ Male □ Female

Experience in teaching science: ___ years

Experience in teaching in practical class: ___ years

Section2. The learning environment in the inquiry-based learning cycle class

Statements	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1. Students in the inquiry class demonstrated a greater use of higher-level skills when compared to those in the traditional class.					
2. The inquiry class has been more effective for revealing students' misconceptions than my usual teaching methods.					
3. The inquiry class seems to have been more effective for teaching scientific concepts than my usual traditional teaching methods.					
4. More misconceptions were identified in this inquiry class than in the traditional class.					
5. Students exhibited greater student-student interactions in this inquiry class than in the traditional laboratory class.					
6. Students were generally more manageable and able to design the experiment in the inquiry class than in the traditional laboratory class					

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Statements	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
7. The students in the inquiry class generally asked more questions than those in the traditional class.					
8. The students in the inquiry class seemed to like biochemistry course more than those in the traditional class.					
9. There was more evidence of conceptual change in the inquiry class than the traditional class.					
10. Students in the inquiry class interacted with the materials more than those in the traditional class.					
11. Students in the inquiry class gained a greater understanding of the concepts being taught than those in the traditional class.					
12. Students in the inquiry class showed a greater ability to use science process skills than students in those in the traditional approach.					
13. Students in the inquiry class were generally more motivated than those in the traditional class.					

Please be brief, but thorough, in answering the following questions:

1. Do you feel the inquiry based learning cycle is an effective method for teaching science? Why? Why not?

   ……

   ……

2. What are some difficulties you identified with using the inquiry based learning cycle?

   ……

   ……

3. What are some advantages you found with using the inquiry based learning cycle?

   ……

   ……

4. What are some disadvantages you found with using the inquiry based learning cycle?

   ……

   ……

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5. What would you want to change in this inquiry based learning cycle?

   ……

   ……

6. Was using laboratory proceeding the lecture is useful? Why? Why not?

   ……

   ……

7. Based on your experience, please list some suggestions or tips for using the inquiry based learning cycle in a science classroom.

   ……

   ……

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APPENDIX H

SEMI-STRUCTURED INTERVIEW QUESTIONS

Objective: To obtain information regarding the student's preference and perception of the learning environment

Interview questions

1. How did you learn in this class?
2. Does the learning in this class differ from the other classes? How?
3. Are activities in this class interesting? How?
4. What activities did you like? Why?
5. Does this class stimulate your thinking skill? How?
6. Were the materials, apparatus or instrument for this experiment easy for you use? How?
7. Was the laboratory manual help you to perform experiments? How?
8. Is the topic too easy or too difficult to learn?
9. Is teaching too fast or too slow?
10. Do you know what is expected of you in the class? Please explain.
11. Do you believe the knowledge that the teacher or teaching assistant presents to You? Why?
12. Does the teacher help you to learn? How?
13. Does the teacher encourage you to learn? How?
14. Does the teacher convey enthusiasm about the course? How?
15. Would you like to learn in this class? Why?
16. Would you be willing take another course that uses this teaching and learning strategy? Why?
17. What overall grade (A-E) would you give for the approach used in this teaching? Give your reason

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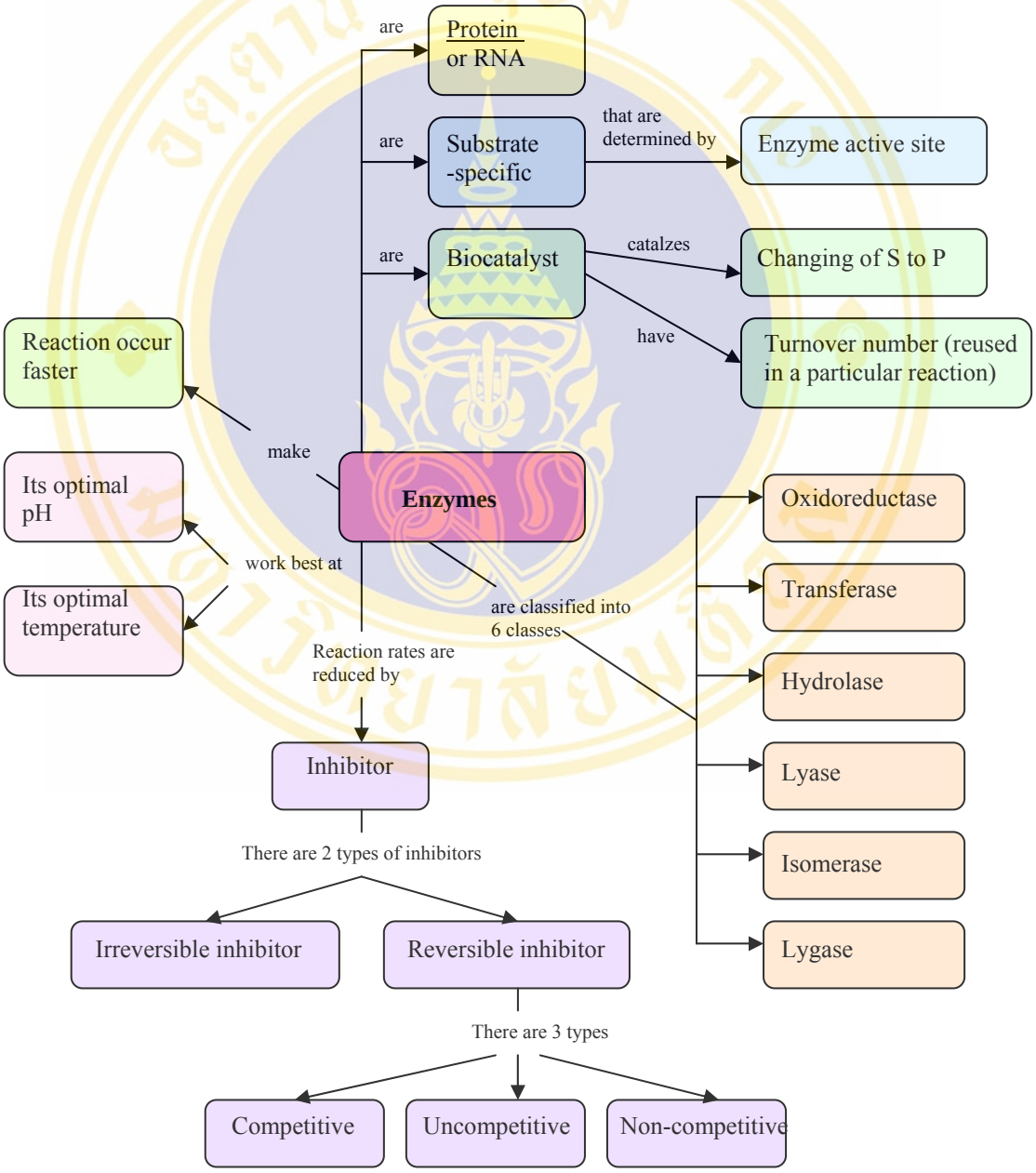
APPENDIX I

EXPERT MAP OF ENZYMES CATALYSIS REACTION



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APPENDIX J

STUDENTS' REASON ON ECAT

Concept 1. Nature of Enzyme

Item	Reason	Respondents (%), N = 72		
		Pre-test	**Post-test** (end of learning cycle class)	**Retention test** (two months after learning cycle class)
1	A	1.4	0.8	0
	B	11.1	7.2	4.2
	C	**54.2**	**87.6**	**85.4**
	D	27.8	4.6	8.3
	No reason	5.5	0	2.1
	Total	100	100	100
2	A	2.8	3.8	4.2
	B	**36.1**	**62.7**	**75.0**
	C	26.4	20.9	16.6
	D	29.2	8.1	4.2
	No reason	5.5	4.6	0
	Total	100	100	100
4	A	0	1.0	0
	B	6.9	14.3	16.6
	C	4.3	7.2	4.2
	D	**81.9**	**76.0**	**77.1**
	No reason	6.9	1.7	2.1
	Total	100	100	100
5	A	1.4	3.6	2.1
	B	**56.9**	**76.5**	**83.8**
	C	8.3	3.2	2.1
	D	4.2	2.5	2.1
	No reason	29.2	14.3	10.4
	Total	100	100	100
6	A	52.8	86.3	87.4
	B	**15.3**	**4.1**	**6.3**
	C	5.5	3.2	0
	D	4.2	3.4	6.3
	No reason	22.2	3.1	0
	Total	100	100	100
7	A	9.7	16.9	27.1
	B	1.4	5.7	14.6
	C	4.2	0	2.0
	D	**50.0**	**59.5**	**43.8**
	No reason	34.7	18.0	12.5
	Total	100	100	100

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Concept 2. How Enzymes Work

Item	Reason	Respondents (%), N = 72		
		Pre-test	**Post-test** (end of learning cycle class)	**Retention test** (two months after learning cycle class)
3	A	13.9	5.2	6.3
	B	9.7	9.0	10.4
	C	8.3	26.7	54.2
	D	**19.5**	**51.4**	**20.8**
	No reason	48.6	7.8	8.3
	Total	100	100	100
8	A	6.9	4.0	2.1
	B	**29.2**	**39.9**	**39.6**
	C	15.3	34.5	39.6
	D	23.6	11.1	18.7
	No reason	25.0	10.6	0
	Total	100	100	100
9	A	4.2	3.1	6.2
	B	**25.0**	**54.7**	**75.0**
	C	4.2	21.6	0
	D	9.7	7.4	6.3
	No reason	56.9	13.4	12.5
	Total	100	100	100
10	A	16.7	36.2	27.1
	B	8.3	9.0	8.3
	C	9.7	7.1	4.2
	D	**43.1**	**40.2**	**50.0**
	No reason	22.2	7.6	10.4
	Total	100	100	100

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Concept 3. Influence of Physical and Chemical Environments on Enzymes' Function

Item	Reason	Respondents (%), N = 72		
		Pre-test	**Post-test** (end of learning cycle class)	**Retention test** (two months after learning cycle class)
11	A	13.9	9.4	20.8
	B	22.2	29.4	16.7
	C	**11.1**	**19.9**	**29.2**
	D	23.6	35.2	27.1
	No reason	29.2	6.3	6.2
	Total	100	100	100
12	A	4.2	3.9	2.1
	B	18.0	21.9	39.6
	C	1.4	0	2.1
	D	**37.5**	**56.9**	**47.9**
	No reason	38.9	17.4	8.3
	Total	100	100	100
13	A	15.3	17.0	18.8
	B	1.4	0.8	10.4
	C	**43.1**	**71.6**	**60.4**
	D	8.3	3.1	4.2
	No reason	31.9	7.6	6.2
	Total	100	100	100
14	A	2.8	2.3	8.3
	B	20.8	51.5	37.5
	C	6.9	1.0	8.3
	D	**55.6**	**38.3**	**41.7**
	No reason	13.9	7.0	4.2
	Total	100	100	100
15	A	8.3	16.5	12.5
	B	4.2	0.8	10.4
	C	**25.0**	**71.6**	**56.3**
	D	8.3	0.8	10.4
	No reason	54.2	10.4	10.4
	Total	100	100	100
16	A	4.2	8.0	10.4
	B	**43.1**	**76.3**	**64.6**
	C	6.9	2.6	6.3
	D	25.0	8.6	14.6
	No reason	20.8	4.6	4.1
	Total	100	100	100

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Concept 4. Influence of Physical and Chemical Environments on Enzymes' Function (Cont.)

Item	Reason	Respondents (%), N = 72		
		Pre-test	**Post-test** (end of learning cycle class)	**Retention test** (two months after learning cycle class)
17	A	5.6	5.7	10.4
	B	36.1	44.0	47.9
	C	1.4	9.6	2.1
	D	**20.8**	**37.7**	**35.4**
	No reason	36.1	3.1	4.2
	Total	100	100	100
18	A	2.8	0.8	4.2
	B	8.3	20.6	12.5
	C	16.7	10.2	10.4
	D	**31.9**	**56.2**	**62.5**
	No reason	40.3	12.3	10.4
	Total	100	100	100

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APPENDIX K

STUDENTS' SCORE ON ELAT

Skills / Contents	Items	Pre-lab score (%)	Post-lab score (%)	Significant difference
Identify E, S, and P of an enzyme reaction				
Identify enzyme	1.1	94.37 ± 2.3	97.05 ± 1.7	0.57
Identify substrate	1.2	86.62 ± 3.4	89.55 ± 2.9	0.65
Identify product	1.5	18.31 ± 3.8	51.49 ± 5.0	0.00*
Write eqution of enzyme reaction	1.7	30.71 ± 4.4	76.12 ± 4.1	0.00*
	Mean ± SD	**57.50 ± 3.5**	**78.55 ± 3.4**	**0.00***
Interpretation & Conclusions				
Compare	1.3	49.30 ± 5.0	79.85 ± 3.9	0.00*
Conclusion based on the emperical data	1.6	54.93 ± 4.2	62.69 ± 5.0	0.28
	Mean ± SD	**52.12 ± 4.6**	**71.27 ± 4.5**	**0.00***
Set hypothesis				
Set the hypothesis to test the experiment	1.4	46.48 ± 4.4	61.94 ± 4.6	0.15
	Mean ± SD	**46.48 ± 4.4**	**61.94 ± 4.6**	**0.15**
Design experiments				
Identify contol of the experiment	2.1	57.35 ± 3.3	80.36 ± 3.2	0.00*
Identify independent variable of the experiment	2.2	52.21 ± 5.0	67.86 ± 4.6	0.07
Measure enzyme activity	2.3	42.53 ± 4.9	49.09 ± 5.0	0.58
	Mean ± SD	**50.70 ± 4.4**	**65.77 ± 4.3**	**0.03***
Calculation rate of reaction				
Calculation an initial rate of a reaction	3.1	12.93 ± 3.2	21.62 ± 3.7	0.02*
Calculation the reaction rate & Explanation	3.2	6.03 ± 2.1	24.32 ± 9.1	0.00*
	Mean ± SD	**9.48 ± 2.7**	**22.97 ± 6.4**	**0.00***
Analysis & Discussion				
Discuss the influence of temperature of an enzyme reaction	3.3	25.44 ± 3.7	38.16 ± 4.1	0.26
Discuss the influences of some factors on enzyme functions	3.4	20.54 ± 9.1	49.12 ± 7.5	0.00*
Analyze the effect of inhibitor on a particular reaction	4	27.00 ± 8.4	49.50 ± 8.9	0.00*
	Mean ± SD	**24.33 ± 7.1**	**45.59 ± 6.8**	**0.02***

Total pre-test score = 40.10 ± 4.5
Total post-test score = 57.68 ± 5.0

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APPENDIX L

SCORES OF STUDENT CONCEPT MAP

Amount of linking terms in student concept maps

	Concept Maps 1 (written before learning)	**Concept Maps 2** (written at the end of laboratory class)	**Concept Maps 3** (written at the end of lecture class)
Range of total linking terms	0-20	0-14	4-33
Range of accuracy Terms	0-16	0-12	0-31
Range of incorrect linking terms	0-5	0-7	0-6
Range of relevant links with the expert map (%)	0-36.67	0-36.67	0-76.67
Average of relevant links with the expert map (%)	4.84 ± 0.95	7.83 ± 1.04	24.01 ± 2.02

The relevant links mentioned by student in the expert map

	Responses (%)		
	Concept Maps 1 (written before learning)	**Concept Maps 2** (written at the end of laboratory class)	**Concept Maps 3** (written at the end of lecture class)
None (0%)	50.0	34.9	3.7
Small (1-32%)	48.4	63.6	72.2
Partial (33-66%)	1.6	1.5	22.2
Substantial (67-99%)	0	0	1.9
Complete (100%)	0	0	0
	100.0	**100.0**	**100.0**

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APPENDIX M

MEAN SCORES OF STUDENT RESPONSES ON CLES QUESTIONNAIRE

Personal Relevance

Item	Statement	Pre-CLES focus on traditional biochemistry class		Post-CLES focus on the learning cycle class	
		Mean	SD	**Mean**	SD
1 (+)	I learn about the world in relevance to my life.	**3.99**	0.68	**4.13**	0.52
7 (+)	New learning starts with problems about the world in relevance to my life.	**4.04**	0.85	**4.11**	0.63
13 (+)	I learn that how science can be part of my life.	**4.12**	0.72	**4.05**	0.52
19 (+)	I get a better understanding of the world outside of classroom.	**3.94**	0.75	**3.98**	0.68
25 (+)	The concepts learned in this class help me to better understand the world in relevance to my life.	**3.90**	0.69	**4.27**	0.70
30 (-)	What I learn in this class has no relevance to my life.	**3.76**	1.01	**3.52**	1.15
37 (-)	What I learn in this class has no relationship to my life.	**3.91**	1.00	**3.68**	1.28
	Average	**3.95**	0.82	**3.96**	0.78

Scientific Uncertainty

Item	Statement	Pre-CLES focus on traditional biochemistry class		Post-CLES focus on the learning cycle class	
		Mean	SD	**Mean**	SD
2 (+)	I learn that scientific theories are human inventions.	**3.24**	0.82	**4.19**	0.61
8 (+)	I learn that science is influenced by people's values and opinions.	**3.95**	0.83	**3.96**	0.62
14 (+)	I learn that the views of science have changed over time.	**3.43**	0.64	**3.66**	0.67
20 (+)	I learn that the biochemical knowledge can be applied to be used in different ways.	**3.97**	0.69	**4.00**	0.63
26 (+)	I learn that scientific knowledge can be questioned.	**4.10**	0.67	**3.96**	0.72
31 (-)	I learn that science reveals the secrets of nature.	**3.48**	0.67	**3.60**	0.80
38 (-)	I learn that scientific knowledge is beyond doubt.	**3.52**	0.78	**3.49**	1.08
	Average	**3.67**	0.73	**3.84**	0.73

For this scale, the alpha reliability is 0.65. However, when item 38 of this scale is removed, this scale produced a better quality of data (alpha reliability after item 38 is remove was 0.70). Therefore, after item 38 is deleted, this scale produced consistently moderated and acceptable results.

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Critical Voice

Item	Statement	Pre-CLES focus on traditional biochemistry class		Post-CLES focus on the learning cycle class	
		Mean	SD	**Mean**	SD
3 (+)	I can ask the teacher "Why do we have to learn this?"	**3.40**	0.92	**4.00**	0.73
9 (+)	I feel free to question the way I'm being taught.	**2.99**	1.09	**3.88**	0.79
15 (+)	I can complain about the activities that are confusing.	**3.00**	0.85	**3.72**	0.65
21 (+)	I feel free to tell the teacher about anything that stops me from learning.	**3.31**	0.96	**3.77**	0.90
27 (+)	I'm free to express my opinion.	**3.63**	0.92	**3.91**	0.75
32 (+)	I can express my opinion.	**3.03**	0.82	**3.69**	0.81
39 (-)	I feel unable to complain about anything.	**3.31**	0.87	**3.23**	0.92
	Average	**3.24**	0.92	**3.74**	0.79

Shared Control

Item	Statement	Pre-CLES focus on traditional biochemistry class		Post-CLES focus on the learning cycle class	
		Mean	SD	**Mean**	SD
4 (+)	I help the teacher to plan what I'm going to learn.	**2.02**	1.01	**3.31**	0.98
10 (+)	I help the teacher to decide how well my learning is going.	**2.74**	1.05	**3.65**	0.87
16 (+)	I have a say in deciding the rules for classroom discussion.	**2.54**	0.90	**3.71**	0.76
22 (+)	I have a say in designing how much time I spend on an activity.	**2.66**	1.11	**3.52**	0.89
33 (+)	I have a say in deciding what will be done on the test or evaluation.	**2.48**	1.09	**3.49**	1.02
36 (+)	I have a say in deciding what activities I do.	**2.94**	0.97	**3.65**	0.78
40 (+)	I have a say in how my learning is assessed.	**2.74**	0.99	**3.49**	0.92
	Average	**2.59**	1.02	**3.55**	0.89

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Student Negotiation

Item	Statement	Pre-CLES focus on traditional biochemistry class		Post-CLES focus on the learning cycle class	
		Mean	SD	**Mean**	SD
5 (+)	I get the chance to share ideas with other students.	**3.94**	0.84	**4.20**	0.76
11 (+)	I can talk with other students about how to solve problems.	**3.91**	0.89	**4.23**	0.61
17 (+)	I try to make sense of other students' ideas.	**3.74**	0.86	**4.30**	0.66
23 (+)	I ask other students to explain their ideas.	**3.38**	0.98	**4.02**	0.68
28 (+)	Other students ask me to explain my ideas.	**3.13**	0.86	**3.72**	0.83
34 (+)	Other students explain their ideas to me.	**3.31**	0.89	**3.88**	0.75
41 (+)	Other students pay attention to my ideas.	**3.15**	0.76	**3.78**	0.79
	Average	**3.51**	0.87	**4.02**	0.72

Attitude

Item	Statement	Pre-CLES focus on traditional biochemistry class		Post-CLES focus on the learning cycle class	
		Mean	SD	**Mean**	SD
6 (+)	I look forward to the learning activities.	**3.80**	0.83	**4.25**	0.69
12 (+)	The activities used in this class are interesting.	**3.88**	0.86	**4.20**	0.69
18 (+)	The activities in this class make me interested in science.	**3.74**	0.73	**4.05**	0.63
24 (+)	I enjoy the learning activities in this class.	**3.55**	0.81	**4.25**	0.73
29 (-)	I feel confuse.	**2.58**	0.95	**2.88**	0.83
35 (-)	The learning activities in this class are a waste of time.	**4.32**	0.99	**4.09**	1.27
42 (-)	I feel tense about what is occurring in this class.	**3.78**	0.86	**3.91**	1.12
	Average	**3.67**	0.86	**3.95**	0.85

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APPENDIX N

TEACHER'S PERCEPTION OF THE LEARNING ENVIRONMENT MEASURED BY TPLEQ

Statements	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1. Students in the inquiry class demonstrated a greater use of higher-level skills when compared to those in the traditional class.		3			
2. The inquiry class has been more effective for revealing students' misconceptions than my usual teaching methods.		2	1		
3. The inquiry class seems to have been more effective for teaching scientific concepts than my usual traditional teaching methods.		3			
4. More misconceptions were identified in this inquiry class than in the traditional class.		3			
5. Students exhibited greater student-student interactions in this inquiry class than in the traditional laboratory class.	1	2			
6. Students were generally more manageable and able to design the experiment in the inquiry class than in the traditional laboratory class	1	1	1		
7. The students in the inquiry class generally asked more questions than those in the traditional class.	1		2		
8. The students in the inquiry class seemed to like biochemistry course more than those in the traditional class.			3		
9. There was more evidence of conceptual change in the inquiry class than the traditional class.		2	1		
10. Students in the inquiry class interacted with the materials more than those in the traditional class.	1	1	1		
11. Students in the inquiry class gained a greater understanding of the concepts being taught than those in the traditional class.		2	1		
12. Students in the inquiry class showed a greater ability to use science process skills than students in those in the traditional approach.		2	1		
13. Students in the inquiry class were generally more motivated than those in the traditional class.	1	1	1		

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BIOGRAPHY

NAME	Miss Piyachat Jittam
DATE OF BIRTH	14 July 1974
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